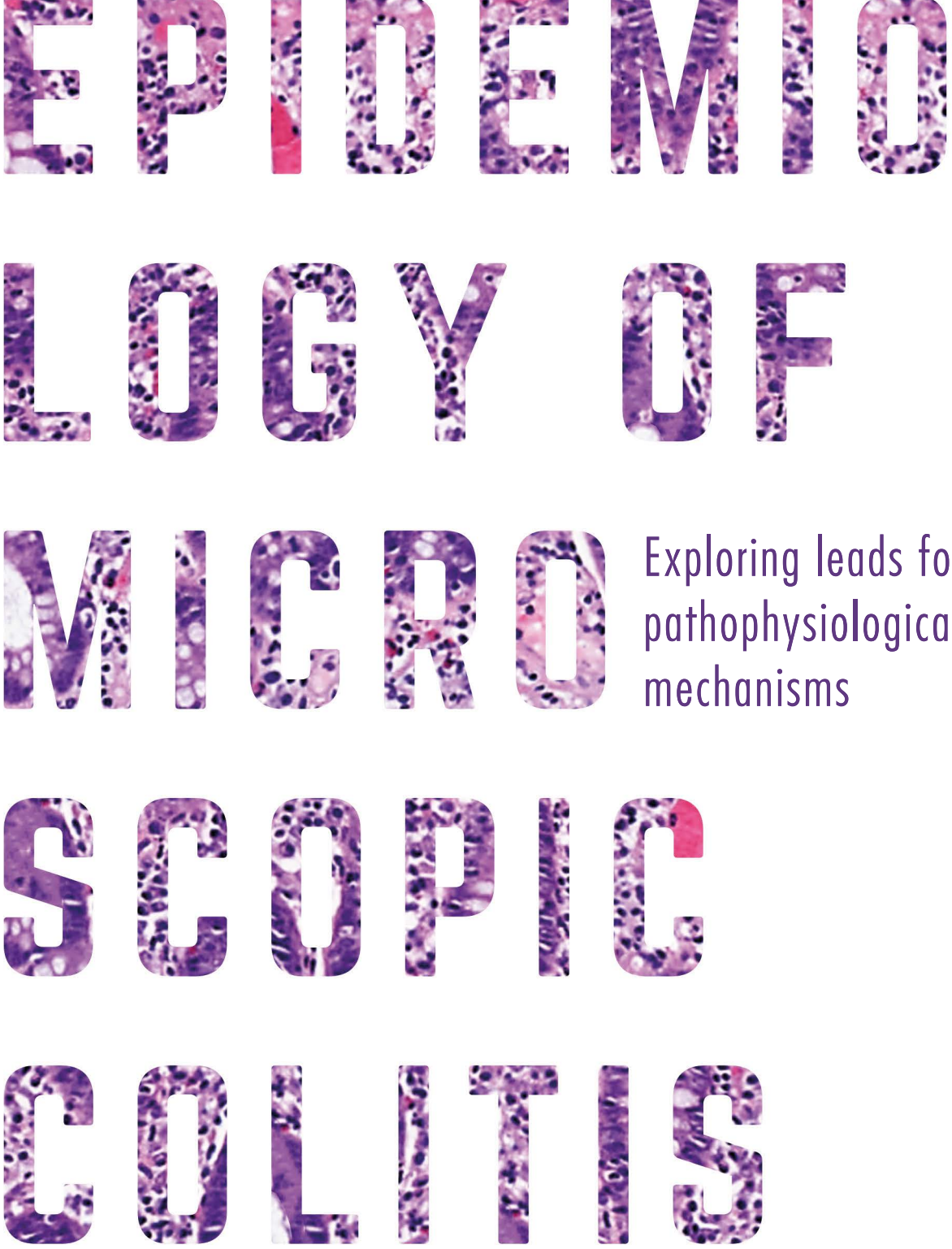
Epidemiology of microscopic colitis



Exploring leads for pathophysiological mechanisms

BAS VERHAEGH

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Epidemiology of microscopic colitis: exploring leads for pathophysiological mechanisms

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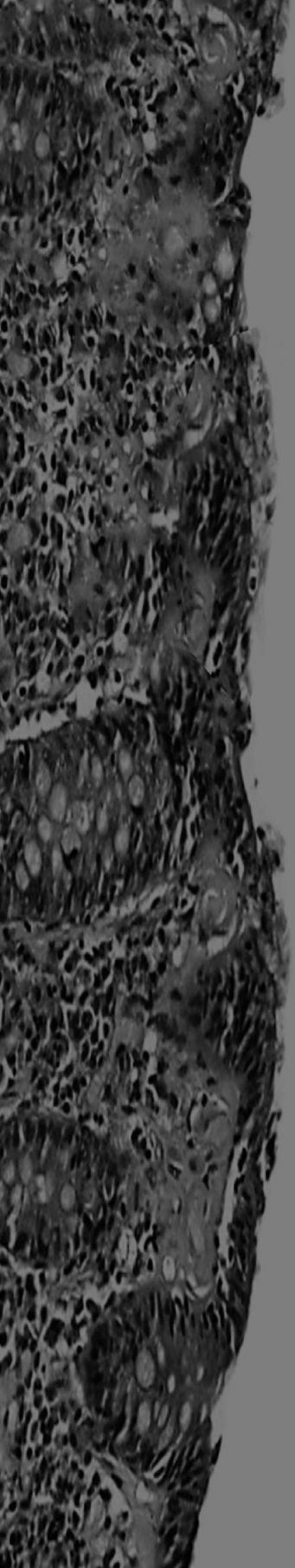

1

General introduction

General introduction

With a prevalence of almost 10%, chronic diarrhea is a frequently reported gastrointestinal condition in the aging population.[1] One of the causes of chronic diarrhea is microscopic colitis (MC), which is diagnosed in about 10-15% of patients with chronic diarrhea referred for colonoscopy MC.[2] Because the world population is aging, the interest in MC has increased over the last decades.

Microscopic colitis

In 1976, Lindström *et al.* published a case of chronic diarrhea with a thickened subepithelial collagen band as primary histological abnormality, establishing the term 'collagenous colitis' (CC).[3] Four years later, the term 'microscopic colitis' was introduced by Read *et al.*, who reported a mild increase of inflammatory cells in colon biopsies of patients with chronic diarrhea and a normal appearance of the colonic mucosa.[4] As an increased number of intraepithelial lymphocytes was a prominent feature, the initial term MC was changed into 'lymphocytic colitis' (LC) in 1989.[5] It lasted until the early '90s before 'microscopic colitis' was reintroduced as an umbrella term for CC and LC, covering any case of chronic diarrhea with histological, but without endoscopic abnormalities.[6]

Histology is the diagnostic hallmark in MC. In both CC and LC, chronic inflammation is present in the lamina propria. For the diagnosis of CC, a thickened subepithelial collagen layer of ≥10µm, containing entrapped capillaries and inflammatory cells should be present. The diagnosis of LC requires an increased number of IELs >20/100 epithelial cells, with a subepithelial collagen layer <10µm. Histological changes not completely fulfilling the criteria for either CC or LC are classified as incomplete MC (MCi).[7] MC is present throughout the entire colon but may show a patchy distribution. There has been a lot of controversy whether only left sided biopsies suffice to diagnose MC,[8,9] or whether right sided biopsies are indispensable for a reliable diagnosis.[10,11] Only recently, a study of Rasmussen *et al.* showed that there is generally no discrepancy between left and right sides biopsies.[12] Nevertheless, endoscopic visualization of the entire colon is an essential part of the diagnostic work-up in (elderly) patients with chronic watery diarrhea, in order to rule out or establish other diagnoses.

From a clinical perspective, LC and CC are indistinguishable.[13] Chronic, non-bloody, diarrhea is the main clinical feature of both subtypes. In most cases, the diarrhea is of watery consistency, with a stool frequency of more than 5 times a day.[9,14] Beside diarrhea, about half of the patients report mild abdominal pain and/or weight loss,[15,16] leading to a symptomatic overlap with diarrhea-predominant irritable bowel syndrome (IBS-D) in about half of the patients.[17,18] Although mucosal biopsies are required to differentiate between MC and IBS, several features have been found to be more

predictive for MC, *i.e.* age >50 years, weight loss, nocturnal stools, recent introduction of a new drug, and the presence of a known autoimmune disorder.[19]
Information on the disease course is mainly derived from retrospective cohorts. Qualitative, prospectively collected data derived from reasonable size populations are lacking. The cohort studies show that the onset of the disease is sudden in up to 42% of patients[15] and the disease course is generally intermittent (65-89%) or continuous in a subgroup (7-13%).[15,20] Spontaneous remission has been reported in up to 15% of patients.[21] In the majority of patients, a multiannual disease course precedes lasting clinical remission.[22,23] Oral budesonide, is the appropriate treatment modality to induce clinical remission.[24] Although 80% of patients respond to budesonide within 6 weeks, the vast majority (*i.e.* 60-80%) has a relapse of symptoms after cessation of treatment.[25,26] Therefore, budesonide maintenance therapy is often warranted.[27] About 10-20% of all patients turn out to be budesonide non-responders, but the reason for this is not yet clear. For this subgroup, no evidence-based treatment alternatives are available, resulting in application of other inflammatory bowel disease (IBD)-derived treatment strategies with varying results. Nevertheless, MC is considered a benign condition. The risk of colorectal cancer or any other malignancy is not increased when compared to the general population[28,29] and MC-related complications predominantly consist of a only few case-reports of endoscopy-related colon perforations.[30] Nevertheless, the chronic and watery diarrhea causes a significant disease burden for the MC patient, as shown by the generally impaired health-related quality of life.[31]

Epidemiology

With a median age at diagnosis of 62 and 65 years for LC and CC, respectively, MC predominantly affects the elderly.[32] Moreover, MC is more frequent in women than in men. Female:male ratios range between 3:1 and 9:1, and are higher in CC compared to LC.[33]
Since the mid-'90s, several population-based epidemiological studies on the incidence of MC have been published.[34-43] Although the majority originates from Europe and North-America, incidence rates from other parts of the world have also been published.[44-46] World-wide, pooled incidence rates of 4.1 and 4.9 per 100,000 person years have been reported for CC and LC, respectively.[32] However, most recent figures show a considerable variation both within and between geographic regions. In Spain, mean annual incidence rates of 2.9 (CC) and 2.3 (LC) per 100,000 person years[40] have been reported between 2004 and 2008. In contrast, Danish data from a comparable period showed incidence rates of 10.8 and 6.7 per 100,000 person years for CC and LC, respectively.[9] Incidence data from the United States were even higher, being 9.1 and 12.0 per 100,000 person years, for CC and LC respectively.[43] So far, only two of all epidemiological studies represented nationwide data and incidence data for the Netherlands are not available.

Interestingly, most studies on incidence data report a clear change over time. For example, Danish data showed incidence rates to increase from 2.9 to 14.9 and 1.7 to 9.8 per 100,000 inhabitants for CC and LC, respectively.[38] Several factors can be cited to explain the gradual increase in MC incidence, such as improved access to colonoscopies and an increased tendency to take biopsies from a normal colon mucosa in the older population.[38] Different age distributions of the background populations might also be a contributive factor. However, an increased awareness for the condition among gastroenterologists and pathologists seems to be an important factor. Nevertheless, a true increase in MC incidence, *e.g.* due to a change in MC associated risk factors, cannot be excluded.

Etiology and pathophysiology

The exact pathophysiological mechanism underlying MC is not clear. MC is considered to represent specific mucosal responses to various luminal agents in genetically predisposed individuals, eliciting an uncontrolled immune response.[47] Therefore, MC is considered a multifactorial disease with genetic, immunological, microbial en environmental factors to be involved. Permeation of luminal substances (*e.g.* drugs, bile acids, nicotine particles, microbial compounds or yet undefined environmental factors) may induce submucosal inflammation and subsequent overt diarrhea. This requires the presence of a disrupted epithelial barrier, which has been found to be present in one *ex vivo* study.[48] Moreover, the hypothesis that luminal agents might trigger inflammation in MC, is supported by observations on resolution of symptoms after fecal stream deviation.[49,50] When bowel continuity was restored, symptoms and inflammation reappeared. The potential MC pathophysiology shows overlap with IBD, IBS and other intestinal inflammatory disorders, but clear evidence underlying these hypotheses in MC is however still limited.

Genetics

A limited number of studies have tried to explore a genetic predisposition in MC patients. As a result, some factors have been linked to MC. For instance, polymorphisms in genes encoding for the HLA-gene and interleukine-6 (IL-6) have been reported to be more prevalent in MC compared to non-MC.[51] IL-6 is a potent pro-inflammatory cytokine, involved in the shift from acute to chronic inflammatory responses.[52] Furthermore, it has pro-fibrotic properties by enhancing collagen production and deposition. The HLA-polymorphisms and especially celiac disease related haplotypes (HLA-DQ2 and DQ1,3) were also found significantly more often in MC patients (35-48%) compared to healthy controls (18-25%).[53-56] Because the main function of HLA class II molecules is to present peptide antigens to T-cells, initiating an immune response, it is

speculated that antigens in the colon luminal may trigger the HLA-modulated inflammatory reaction.
Furthermore, polymorphisms in matrix metalloproteinase related genes[57] and serotonin reuptake transporter have been reported.[58] Most of these studies published so far are based on rather small numbers of MC patients and mainly concern CC specifically. Large genome-wide association studies in MC are therefore needed.

Immunology

As MC is an inflammatory condition, the immune system is likely to be involved. Although research data on the involvement of the innate immunity in MC pathophysiology are limited, its role is generally assumed to be only minor due to lack of granulocyte infiltrates[7] and the low diagnostic value of granulocyte-derived proteins as lactoferrin and calprotectin.[59] Recent studies, evaluated the role of the adaptive immune system in MC (60-64) and found that LC patients exhibit more $CD8^+$ and less $CD4^+$ and $CD4^+/CD8^+$ cells, compared to CC and non-MC controls.[62,64] Furthermore, an increased mucosal mRNA expression of Th1 and Th17 related proteins was reported, presuming a mixed Th1-Th17 response in MC.[63] However, protein levels of Th1 and Th17 related cytokines were not increased.[64] Moreover, higher protein and mRNA levels of the anti-inflammatory cytokine IL-10 have been reported in MC, which is produced by regulatory T-cells.[60,64] Due to their immunosuppressive mechanism of action, increased numbers of these cells are also found in some autoimmune disorders. Possibly, the increased levels of IL-10 keep the degree of inflammation to a relative minimum in MC.[64] Altogether these findings give leads for a dysregulation of the adaptive immune response in MC, which matures by environmental microbial exposure in the first years of life. Despite the current findings, immunological studies in MC are still in its infancy.

Microbiota and environmental factors

Although luminal factors are thought to be involved in the MC pathogenesis, the role of microbial and environmental factors has not been studied. Only one brief report has been published, showing that the number of *Akkermansia spp* in MC is decreased compared to non-MC controls.[65] As these bacteria were found to be associated with the thickness of the colon mucus layer in mice, decreased numbers of *Akkermansia spp* might lead to a thinner mucus layer, potentially enhancing mucosal exposure to toxic fecal substances.
With regard to environmental factors, only one study has been published, showing that MC is more common in smaller cities, with predominantly white residents and lower annual incomes.[66] Whether the exact home address and the corresponding exposure to environmental factors (*e.g.* pollution) is of influence on the risk of MC, has never been assessed.

Risk factors

As the pathophysiology of MC is not yet clear, several studies tried to identify risk factors, which may provide leads for underlying mechanisms. First, concomitant autoimmune disorders (*e.g.* rheumatoid arthritis, thyroiditis, celiac disease) are frequently reported,[16,67] suggesting an autoimmune component in the MC pathogenesis. In addition to the increased prevalence of celiac disease related HLA-DQ2 or DQ8 haplotypes, serological or histological features of celiac disease are estimated to be present in 5-15% of MC patients.[68-70]

MC has also been found to be associated with bile acid malabsorption (BAM) in about 45% of MC patients.[71-73] A reduced capacity of the ileum to absorb bile acids is assumed to be the underlying pathophysiological mechanism, rather than chronic malabsorption due to diarrhea. The reduced absorptive capacity of the ileum might be due to inflammation, reducing the expression of active sodium bile acid transporters (ASBT). As a consequence, unabsorbed bile acids may be able to increase paracellular permeability and alter the intestinal microbiota composition and activity. Oral budesonide, the treatment of first choice in MC, upregulates the ileal expression of ASBTs.[74] In theory, this would reduce colon bile acid exposure and restore paracellular permeability to normal. However, only 55-75% of MC patients exhibit signs of ileal inflammation.[75] Therefore, BAM might be involved in MC pathogenesis, but is not the sole cause.

Cigarette smoking has also been found to be positively associated with MC. Current smokers have a 3-5 times increased risk of developing MC.[76,77] Furthermore, smokers tend to develop their disease at least 10 years earlier compared to non-smokers.[77,78] There is however no evidence that smoking negatively impacts clinical symptoms or remission rates after treatment.[78] Although current, active smoking is strongly associated with MC, the impact of passive nicotine exposure and underlying mechanisms on the MC risk have never been established. Other lifestyle factors have hardly been studied. Two studies have addressed the role of alcohol exposure, but with contradictive results[77,79] and one study addressed the role dietary factors in MC but found no clear associations.[80]

Drug consumption

Drug use has repeatedly been associated with an increased risk of MC. Based on published case series of so-called 'drug-induced MC', non-steroidal anti-inflammatory drugs (NSAIDs), proton-pump inhibitors (PPIs) and selective serotonin reuptake inhibitors (SSRIs) were among the drug classes with a high likelihood of triggering MC.[81] These associations were later confirmed in case-control studies.[82-84] However, many of these studies suffer from methodological limitations, such as a self-reported nature of drug use,[82] classifying a once-only prescription as positive exposure,[83,84] or disregarding a latency period between drug exposure and the actual diagnosis.[82-84] A recent study confirmed the association between MC and exposure to NSAIDs or PPIs, by applying

non-MC colonoscopy patients as controls.[85] Nevertheless, the exposure characteristics (based on duration, recency and dosage of exposure) exhibiting the highest risk of MC have never been assessed systematically, which would be of relevance to better identify subjects with a possible drug-induced MC in daily clinic. In addition, the impact of NSAID and PPI co-exposure has never been studied either.

The proportion of MC patients with a drug-induced cause of the disease is unknown, but up to 35-55% of patients have been reported to use PPIs or NSAIDs in the year before diagnosis.[82,83] To strengthen the assumption of a drug-induced cause, the timing of exposure with respect to the start of symptoms is of relevance. Withdrawal of the suspected drug should relief diarrheal symptoms, whereas re-challenge of the drug should lead to symptom recurrence. Furthermore, it should be noted that both NSAIDs and PPIs are widely used and only a small proportion of subjects develops MC. This raises questions on the possible underlying pathophysiological mechanisms and the role of host-susceptibility.

Currently, the mechanisms by which NSAIDs and PPIs trigger MC-related inflammation are not fully understood. Unfortunately, recent studies on drug-induced MC do not address possible mechanisms, and merely hypothesize on their existence. Based on these hypotheses, it is presumed that these drugs might affect the colon mucosa directly, *e.g.* by affecting tight junction structures, or indirectly, by changing the luminal environment, which might predispose to a local inflammatory reaction in turn. Nevertheless, given the widespread use of NSAIDs and PPIs and the relatively low incidence of drug-induced MC, an idiosyncratic drug reaction in genetically predisposed hosts cannot be excluded.[86]

Aims and outline of this thesis

Epidemiological studies generally show rising incidence rates of MC over the last two decades. Although the number of studies on MC etiology and pathophysiology has grown extensively, the set of currently studied risk factors for MC is relatively limited. For instance, underlying mechanisms of drug-induced MC or the role of environmental factors have barely been explored. Therefore, this thesis aims to study the incidence and clinical characteristics of MC in the Netherlands, to explore current and potential new risk factors, and to find new leads for underlying pathophysiological mechanisms, by applying epidemiological studies.

First, we performed an epidemiological study on the incidence of MC in the Netherlands between 2000 and 2012 (Chapter 2). This is of relevance as nationwide data from western-European countries, and the Netherlands in particular, are lacking. Possible explanations for the rising incidence rates over time will be discussed.

In Chapter 3 the clinical characteristics of MC patients from the southeastern part of the Netherlands are described. Cases were derived from the national MC population, reported in the previous chapter. Various aspects of the population will be addressed, including demographics, clinical data and treatment regimens applied. This population has served as the base for the other studies in this thesis.

Although MC is considered a disease with a multifactorial etiology, the variation in the currently studied risk factors is rather limited. Besides demographic (age, gender) and clinical factors (concomitant diseases), the only other frequently studied risk factors for MC are smoking and drug exposure. In would be of clinical relevance to obtain more detailed insight in exposure characteristics of MC associated drugs. They may give leads for potential underlying pathophysiological mechanisms and enable to outline the risk profile of MC cases in which drug-induced cause is expected. In Chapter 4, a case-control study on drug-induced MC is presented, exploring the effect of dosage, recency of exposure and the duration of continuous use on the risk of MC. Furthermore, we evaluated epidemiological data on drug-exposure to find leads for possible underlying mechanisms. As observational data do not prove causality, *in vitro* and *ex vivo* experiments were then conducted to assess a possible direct effect of MC associated drugs on the epithelial barrier function of the colon. The *ex vivo* experiments were also performed to explore involvement of host-susceptibility for drug-induced MC. The results of this study are reported in Chapter 5.

According to current insights, mucosal responses to various luminal agents, eliciting an uncontrolled immune response are considered a key step in MC pathophysiology. The maturation and functioning of the immune system is imprinted by early life exposure to microorganisms. In Chapter 6, factors associated with an enhanced exposure to microbial agents in early life were evaluated. Moreover, other known and yet unknown environmental factors were studied. In a separate study, the effect of exposure to polluting sources was assessed. After all, increased exposure to *e.g.* air pollution has been associated with several gastrointestinal disorders, such as IBD. Chapter 7 describes a case-control study, in which we used GPS coordinates to assess the effect of environmental factors present in the residential area on the risk of MC.

While conducting the studies comprised in this thesis, it became clear that systematically recorded epidemiological and clinical data on MC are lacking. As part of the European Microscopic Colitis Group (EMCG), we therefore established a prospective, European registry for MC, called the PRO-MC Collaboration (Chapter 8*).*

Finally, a general discussion is presented in Chapter 9, summarizing the major findings and discussing remaining questions and potential implications for clinical practice and future research.

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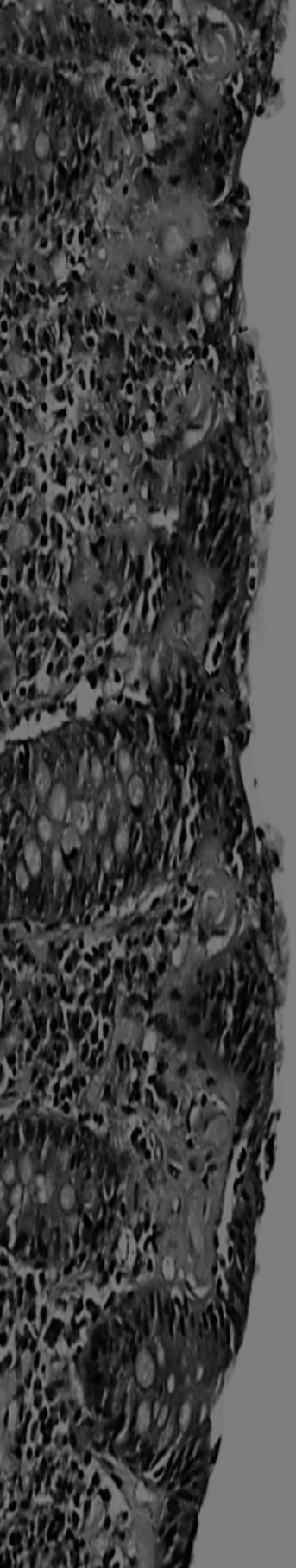

2

Incidence of microscopic colitis in the Netherlands. A nationwide population-based study from 2000-2012

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Abstract

Background

Incidence rates of microscopic colitis are mainly based on regional data from a limited number of countries. To evaluate geographical differences and changes over time, more nationwide incidence rates are needed. The aim of this retrospective study was to assess the incidence rate of microscopic colitis in the Netherlands in a nationwide cohort.

Methods

A search was performed in the Dutch pathology registry, covering records of all approximately 16.5 million inhabitants. Incident cases were defined as a first diagnosis of microscopic colitis (collagenous or lymphocytic colitis) between 2000-2012.

Results

In total, 7,228 incident cases were identified with a mean annual incidence rate of 3.4 per 100,000 person years. Collagenous colitis was present in 3,741 cases and lymphocytic colitis in 2,718 cases, with a mean annual incidence rate of 1.8 and 1.3 per 100,000 person years, respectively. Remaining 769 cases were described as undefined microscopic colitis. Collagenous and lymphocytic colitis incidence rates increased significantly over time ($p<0.001$) with a male:female ratio of 1:3 and 1:2, respectively.

Conclusion

The Dutch mean annual incidence rates of collagenous and lymphocytic colitis were considerably lower than previously reported by other countries. However, incidence rates increased gradually over time, with a clear female predominance.

Introduction

Microscopic colitis (MC) is an umbrella term for chronic watery diarrhoea with a normal endoscopic appearance of the colonic mucosa, and characteristic histological abnormalities. MC includes two subtypes, *i.e.* collagenous colitis (CC) and lymphocytic colitis (LC).[1,2] Histologically, the hallmark of CC is the presence of a thickened subepithelial collagen layer (10 µm or more), whereas LC is characterized by an increased number of intraepithelial leukocytes.[3-5] Both MC entities are predominantly found in females over 65 years of age.[6] Whether CC and LC can be considered histopathological variants of the same disease or two different clinical entities is still matter of discussion.[7-9] Clinically, both CC and LC are characterised by a chronic relapsing course.[10,11] Although MC is successfully treated with oral budesonide in most cases, the relapse rate after cessation of treatment is 50-80%.[12] The chronic, relapsing, watery diarrhoea is the main contributor to the significantly decreased quality of life in MC patients.[13-15]

Epidemiological studies performed in Sweden reported mean annual incidence rates up to 5.4 per 100,000 person years for CC in 2005-2010[16] and 4.5 per 100,000 person years for LC in 2004-2008.[17] Data collected in the US (Olmsted County, Minnesota) between 2002-2010, showed incidence rates of 7.1 and 9.5 per 100,000 person years for CC and LC, respectively,[18] while a recent Spanish study[19] reported incidence rates of 2.6 and 2.2 per 100,000 person years between 2004-2008 for CC and LC, respectively. Most of the epidemiological studies described an increase in incidence rates over time,[12,19-21] but recent follow-up data of two long-term, regional cohorts showed a stabilization of the incidence rates of both CC and LC over the last 10-15 years.[17,18] Although differences between countries seem to be present, the number of studies is too limited to draw firm conclusions on the geographical distribution of MC and changes over time.

The majority of epidemiological studies reporting MC incidence rates were based on regional data with populations up to 650,000 inhabitants.[16-27] Only one nationwide study was published so far, covering a rather small population of 277,184 inhabitants.[28] To evaluate potential geographical difference and changes over time, and to estimate the disease burden of MC in future, more data on long-term, nationwide incidence rates are warranted. Therefore, the aim of the present study was to assess the mean annual incidence rate of MC in the Netherlands in a nationwide, population-based cohort, spanning a thirteen-year period.

Materials and methods

Catchment area

Data on age, size, and sex distribution of the Dutch population between January 1, 2000 and December 31, 2012, were obtained via Statistics Netherlands (*www.cbs.nl*). In this time period, the total population increased with 5.8%, from 15,863,950 inhabitants in 2000 to 16,778,025 in 2012. The age and sex distribution in 2000 and 2012 is shown in Figure 2.1.

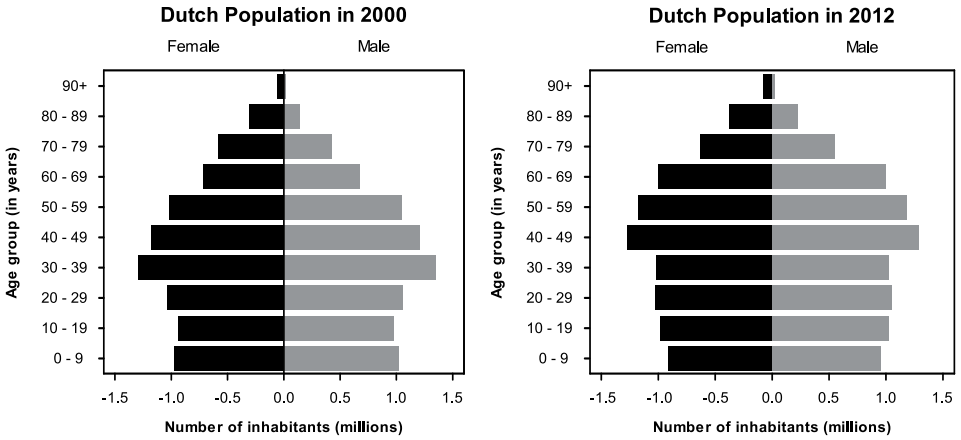

Figure 2.1 Age pyramid of the Dutch population at December 31, 2000 (left) and December 31, 2012 (right)

Pathology registry

The Netherlands has 55 pathology departments on a total of 132 hospitals, of which eight are university hospitals. Each pathology report generated in the Netherlands is archived in the nationwide registry of histo- and cytopathology, called PALGA.[29] This database was established in 1971 by the PALGA-foundation and reached full national coverage from 1991 onwards. Pathology reports are received on a daily basis and are automatically transposed into standardised excerpts containing encrypted patient data, a pathologist's conclusion, and a PALGA-diagnosis based upon the Dutch version of the Systemized Nomenclature of Medicine (SNOMED).[29]

Patient identification

For this retrospective study, a search was performed in the PALGA registry, using the following search terms: microscopic colitis, collagenous colitis, and lymphocytic colitis. The initial selection included any subject with at least one excerpt, generated between

January 1, 2000 and December 31, 2012, in which the term MC, CC, or LC was mentioned. Subsequently, all registered colon biopsies of each subject meeting the search criteria were added in order to ascertain selection of incident cases only. The process of patient identification is schematically presented in Figure 2.2. Excerpt evaluation was performed by a trained investigator (BV) after agreement on the diagnostic criteria with an experienced pathologist (AD). Patients' sex, age, and year of diagnosis were recorded. The year of diagnosis was defined as the year in which a positive diagnosis of CC, LC, or undefined MC (uMC) was reported for the first time. Incident cases were defined as a first diagnosis of CC, LC, or uMC between January 1, 2000 and December 31, 2012. Each incident case was included only once, regardless of a change in MC subtype or a suggested recurrence in later biopsies.

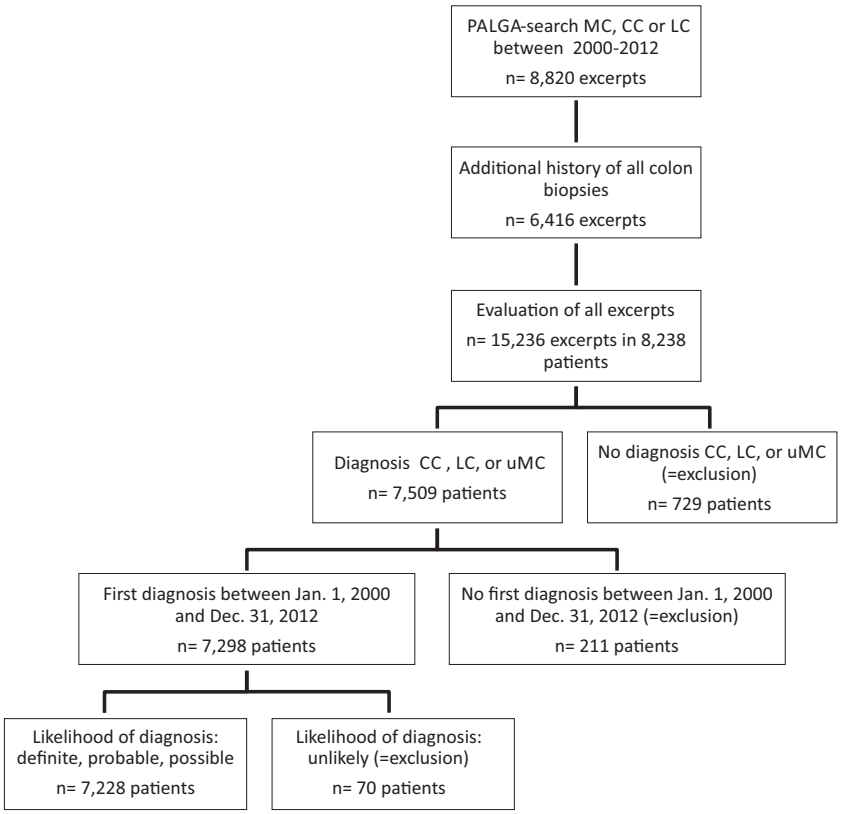

Figure 2.2 Sequence of patient identification. MC: microscopic colitis; CC: collagenous colitis; LC: lymphocytic colitis; uMC: undefined microscopic colitis; PALGA: Dutch registry of histo- and cytopathology

Diagnostic criteria

The generally accepted diagnostic criteria for CC and LC were applied.[6] Besides chronic inflammatory changes in the lamina propria and a damaged surface epithelium, this included a thickened subepithelial collagen layer (≥10µm) for CC and an increased number of intraepithelial leukocytes (IEL ≥20/100 epithelial cells) without a thickened collagen layer for LC.[2,5] If no information for further subtyping was available, cases were classified as uMC. This term was chosen to avoid any confusion with the term 'incomplete microscopic colitis', which is used for patients with typical MC symptoms not fulfilling the strict histologic criteria for either CC or LC.[25] When features of both CC and LC were mentioned, cases were classified as the subtype most prominently present. However, PALGA excerpts consist of a pathologist's conclusion solely, frequently leaving specific diagnostic criteria unmentioned. Therefore, the likelihood of the diagnosis was determined for each evaluated excerpt, based on the pathologist's description. Together with an experienced pathologist (AD), the probability to comply with the diagnosis was defined as definite, probable, possible, or unlikely. Cases meeting the specific diagnostic criteria, or cases described as *e.g.* 'compatible with CC' or 'may very well fit LC' were defined as definite. Cases described as *e.g.* 'suspicious for CC', 'considered LC', 'early phase of CC' were defined as probable. Cases were defined as possible when terms like 'a suggestion of CC', 'some signs of LC' or 'a slight indication of CC' were given. Remaining cases were classified as unlikely.

Colonoscopy rate

Information on the total number of colonoscopies and flexible sigmoidoscopies annually performed in the Netherlands was available for the years 1999, 2004, 2009, and 2011.[30-33] These data were based on surveys sent to all Dutch endoscopy units.

Statistics

Demographic data were presented as median and inter-quartile range (IQ, 25^{th} – 75^{th} percentiles). Incident cases were defined as all subjects with a first time definite, probable, or possible diagnosis of LC, CC, or uMC between January 1, 2000 and December 31, 2012. Cases defined as unlikely to comply with the diagnosis were excluded from any further analysis. Mean annual incidence rates with 95% confidence intervals (CI) were calculated by dividing the number of incident cases in a particular year by the total number of inhabitants on December 31 of that year. Age-adjusted incidence rates were calculated based on the age distribution of the population on December 31 of a specific year. For incidence calculations, a Poisson distribution of the incident cases per year was assumed. Incidence rate ratios (IRR) were calculated to compare incidence rates (*e.g.* male versus female, and CC versus LC) at a certain time point. Linear regression analysis was applied to determine whether an increase in

incidence rate or IRR over time was significant. All statistical evaluations were performed with IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

The study was approved by the Medical Ethical Committee of Maastricht University Medical Center+, Maastricht, the Netherlands.

Results

Patients

A total number of 15,236 excerpts (8,820 initial selection and 6,416 additional colon reports), belonging to 8,238 patients, were extracted from the PALGA registry. After evaluation of all excerpts, 7,509 MC patients could be identified of which 7,298 patients were diagnosed with MC for the first time between January 1, 2000 and December 31, 2012 (Figure 2.2). Patient characteristics, as well as the distribution of the four probability categories are listed in Table 2.1. A total number of 70 cases were defined as unlikely and therefore excluded from further analyses. All numbers reported below are based on the incident cases classified as possible, probable, and definite (n=7,228).

Table 2.1 Patient characteristics

	CC	LC	uMC	Total
Total (n)	3,768	2,741	789	7,298
Sex (% female)	75.6%	70.3%	64.1%	72.4%
Age (years)				
Minimum	14	1	12	1
Maximum	96	96	95	96
Median (IQ)	61 (51-72)	61 (49-71)	59 (47-72)	61 (49-71)
Median Men (IQ)	61 (49-71)	62 (50-71)	59 (47-70)	61 (49-71)
Median Women (IQ)	62 (52-72)	61 (49-71)	59 (47-72)	61 (50-72)
Probability				
Unlikely	0.7%	0.8%	2.5%	0.9%
Possible	14.7%	13.8%	22.7%	14.8%
Probable	20.8%	22.0%	23.6%	21.9%
Definite	63.8%	63.3%	51.2%	62.4%

CC: collagenous colitis; LC: lymphocytic colitis; uMC: undefined microscopic colitis; IQ: interquartile range

The total group of MC patients identified between 2000 and 2012 showed a male:female ratio of 1:2.6 and a median age at diagnosis of 61 (50-71) years. Mean annual incidence rates of MC for the total study period 2000-2012 were 3.4 (95% CI 3.3-3.5) per 100,000 person years; 1.9 (95% CI 1.8-2.0) per 100,000 person years for men and 4.9 (95% CI 4.7-5.0) per 100,000 person years for women. The mean annual incidence rates

increased significantly in both sexes, from 1.0 to 2.9 per 100,000 person years in men (p<0.001) and from 2.8 to 7.9 per 100,000 person years in women (p<0.001) (Figure 2.3A-C). Calculation of IRRs showed that the increase in female incidence rates over time was not significantly higher compared to the increase in males (p=0.81).

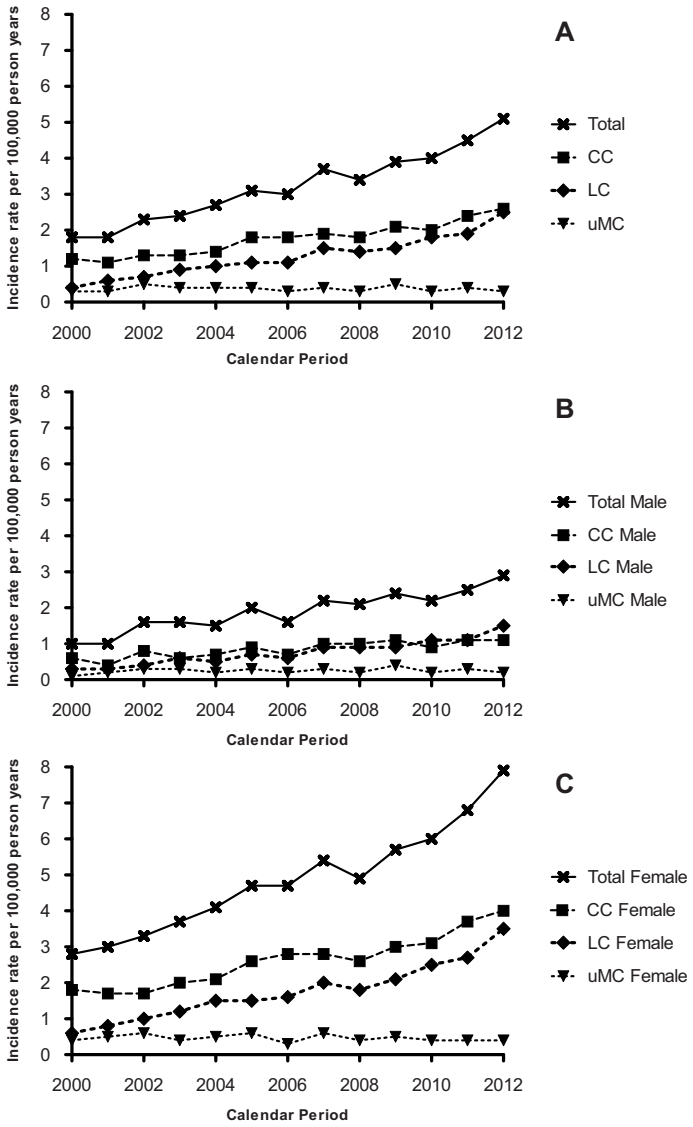

Figure 2.3 Age-adjusted, mean annual incidence rates for both sexes (A), and males (B) and females (C) separately between 2000 and 2012

Collagenous colitis

Between January 1, 2000 and December 31, 2012, CC was diagnosed in 3,741 patients, including 904 males and 2,837 females (ratio 1:3.1). Details on age and likelihood of the diagnosis are listed in Table 2.1.
The mean annual incidence rate of CC was 1.8 (95% CI 1.7-1.8) per 100,000 person years for the total study period (i.e. 2000-2012), being 0.9 (95% CI 0.8-0.9) per 100,000 person years for men and 2.6 (95% CI 2.5-2.7) per 100,000 person years for women. During the thirteen-year period, the mean annual incidence rate gradually increased from 1.2 to 2.6 per 100,000 person years ($p<0.001$). The mean annual incidence rate increased from 0.6 to 1.1 per 100,000 person years in men ($p<0.001$) and from 1.8 to 4.0 per 100,000 person years in women ($p<0.001$) (Figure 2.3B, 2.3C). This increase did not differ significantly between sexes ($p=0.68$).

Lymphocytic colitis

Between January 1, 2000 and December 31, 2012, LC was reported in 2,718 patients, including 809 males and 1,909 females (ratio 1:2.3). The median age and likelihood of diagnosis are listed in Table 2.1.
For the period 2000-2012, the mean annual incidence rate was 1.3 (95% CI 1.2-1.3) per 100,000 person years, being 0.8 (95% CI 0.7-0.8) per 100,000 person years for men and 1.8 (95%CI 1.7-1.9) per 100,000 person years for women. From 2000 to 2012, mean annual incidence rates increased significantly in the total population, from 0.4-2.5 per 100,000 person years ($p<0.001$). Analysing men and women separately, incidence rates increased from 0.3 to 1.5 per 100,000 ($p<0.001$) and from 0.6 to 3.5 per 100,000 ($p<0.001$) person years, respectively (Figure 2.3B, 2.3C). When compared to males, female incidence rates did not increase significantly more over time ($p=0.51$).
Linear regression analysis of IRRs showed that mean annual incidence rates of LC increased significantly more over time compared to CC incidence rates ($p<0.001$).

Undefined microscopic colitis

A total number of 769 patients; 276 male, 493 female (ratio 1:1.7) could not be classified as either CC or LC and were therefore designated as uMC. More details are provided in Table 2.1. Both general and sex specific mean annual incidence rates of uMC cases between 2000 and 2012 fluctuated around 0.4 (95% CI 0.3-0.5) per 100,000 person years and changed only marginally over time ($p=0.85$) (Figure 2.3A-C).

Age

For both CC and LC, sex specific incidence rates increased significantly with advancing age ($p<0.001$) (Figure 2.4A, B). The highest age adjusted incidence rates for both men and women were found in the age group ‘60-69 years’. All crude incidence rates were

equal to the age adjusted incidence rates. In general, any person aged 65 years or above had a 4.1 times increased risk of CC (95% CI 3.9-4.4) or LC (95% CI 3.8-4.4) compared to those younger than 65 years. Females of 65 years of age or above had a 3.6 (95% CI 3.4-3.9) times increased risk of CC and 3.3 (95% CI 3.0-3.6) times increased risk of LC compared to females below 65 years of age. Similar odds ratios were found for females of 65 years of age or above compared to the whole population.

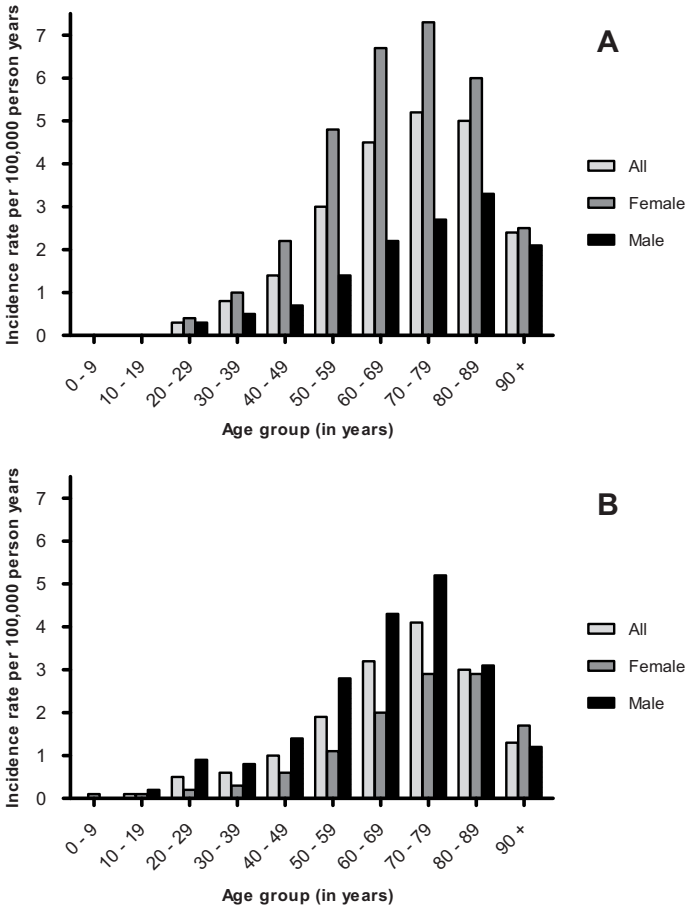

Figure 2.4 Sex and age specific mean annual incidence rates of collagenous (A) and lymphocytic (B) colitis between 2000 and 2012

Annual colonoscopy rate

The total number of colonoscopies and flexible sigmoidoscopies performed in the Netherlands was available for 1999, 2004, 2009 and 2011 and was found to increase

gradually over time (Table 2.2). The number of MC cases per 1,000 colonoscopies increased as well.

Table 2.2 Annual colonoscopy rate in the Netherlands

	Endoscopies (Total)	Colonoscopies, Sigmoidoscopies	Total MC Cases	Rate
1999[a]	325,000		286[b]	
2004	408,982	186,864	436	2.3 / 1,000
2009	505,226	249,233	648	2.6 / 1,000
2011	507,145	258,204	752	2.9 / 1,000

MC: Microscopic colitis; [a] No data on the colonoscopy rate in 2000 were available; [b] Total microscopic colitis (MC) cases in 2000

Discussion

The present nationwide population-based study showed mean annual incidence rates of 1.8 and 1.3 per 100,000 person years for CC and LC, respectively, over the period 2000-2012. The highest age-adjusted incidence rates were reported for females and for subjects between 60-69 years of age. In addition, a significant increase over time was observed for the total group of MC, as well as for CC and LC separately.

The mean annual incidence rates reported in the present study were considerably lower than those in other countries. The only other nationwide study, performed in Iceland, reported incidence rates of 5.2 and 4.0 per 100,000 person years for CC and LC, respectively, between 1995-1999.[28] Considering the time period and the reported increases in MC over time,[18-20] the difference with the Dutch incidence rates might be even larger. Furthermore, in a time period comparable to the present study, a regional Swedish study reported an incidence rate of 5.4 per 100,000 person years for CC[16] and a Spanish study presented incidence rates of 2.9 and 2.3 per 100,000 person years for CC and LC, respectively.[19] Based on all results, a north-south gradient for MC, as proposed in literature,[16,19] cannot be confirmed.
A clear explanation for the low incidence rates is lacking. To assess the completeness of the PALGA-registry for MC, a quality check was performed by a simultaneous search in the pathology registry of the Maastricht University Medical Center, Maastricht, the Netherlands. This showed up to 94% coverage of the PALGA-search. Missing substantial numbers of diagnosed cases is therefore unlikely. Underdiagnosis of MC in the Dutch population however, cannot be ruled out, especially in the first years of our study period. But the awareness for MC among both gastroenterologists and pathologists did increase enormously over the last decade.[34,35] Therefore, the figures in the last years of our study period are likely to reflect the true Dutch incidence rates, although still being low compared to other studies. Inclusion of the small number of subjects (n=70) with an

unlikely diagnosis of MC would not have resulted in higher incidence rates. On the other hand, also a slight overestimation of the number of incident cases cannot be excluded, as about 15% of excerpts were classified as possible. If corresponding biopsy specimens could have been revised, a proportion of these cases might not have met the diagnostic criteria for either CC or LC. Furthermore, clinical information (*e.g.* the presence of chronic watery diarrhoea and the absence of other gastrointestinal pathology leading to an increased IEL count or thickened collagen layer) was not available. The investigators were familiar with the symptomatic overlap between irritable bowel syndrome (IBS) and MC,[36] as well as the presence of MC-like inflammation in a large proportion of IBS patients,[37] but it was not possible to correct for this.

Overall, the Dutch MC incidence rates seem to be lower than in other countries. This international variation is unlikely to be caused by major differences in demographics. Scandinavian populations for example, are relatively comparable to the Dutch regarding age- and gender distribution. Genetic susceptibility and environmental and lifestyle risk factors are more likely contributors to the variation between countries. Moreover, methodological differences such as catchment area, data registration, and data collection were present. International differences in referral policy, awareness, and indications for diagnostics or biopsy taking in patients with chronic diarrhoea may also exist.

In the present study, incidence rates were found to increase with age. The highest age-specific incidence rates for CC and LC were found in 70-79 year old females and 80^{+} year old males, which was comparable to recent epidemiological studies.[16,17,21] However, these age groups represent a relatively small proportion of the total population. Adjusting for age showed that peak incidence rates for CC and LC were actually present in the age group of 60-69 year olds. Male:female ratios for both CC (*i.e.* 1:3.1) and LC (*i.e.* 1:2.3) were in line with literature as well.[16,19] The association between auto-immune diseases and MC, and the higher prevalence of auto-immune diseases in females is a generally accepted hypothesis for the female predominance.[38] Assuming that medication use (*e.g.* NSAIDs, PPIs) is a potential risk factor for MC, this might be an explanation for the MC peak incidences in elderly. However, the exact aetiology of MC is still unclear and these hypotheses lack strong scientific support.

We clearly demonstrated a gradual and significant increase of the mean annual incidence rates for MC as a whole and for CC and LC separately. To exclude that the observed increase was due to changes in the age distribution over time, the age-adjusted incidence rates of 2012 were recalculated with the distribution of 2000. This did not affect the significant increase of mean annual incidence rates. Additionally, sensitivity analyses showed that the increase of mean annual incidence rates over time of the total population, and CC and LC separately, was independent of the probability

categories included. Exclusion of all possible or all possible plus probable cases still showed a highly significant increase of incidences rates over time.
As the annual number of colonoscopies and sigmoidoscopies performed in the Netherlands increased during the studied period, this might have affected the mean annual incidence rates. However, the number of MC cases per 1,000 colonoscopies increased only slightly, which points to a true rise in MC incidence in the Dutch population. Unfortunately, further national or regional data on the annual colonoscopy rate, number of biopsies taken, or colonoscopy indication were not available and therefore the abovementioned findings should be interpreted with care.

Some remarks can be made about the definition of MC.[39] Over the years a general consensus has emerged that MC should be used as an umbrella term covering CC, LC, and some atypical subtypes of chronic watery diarrhoea with a normal appearing endoscopy, not completely fulfilling the histological criteria for either CC or LC.[3,25] Previously, this differentiation was less clear and the term MC was often applied to biopsies with an increased IEL count but without the CC specific collagen band.[1,2] Besides, the clinical characteristics and therapeutic options between LC and CC are indistinguishable. These factors may contribute to a pathologist's motive to define a certain histological presentation as MC, instead of LC or CC specifically.[5,25] This could explain the relatively high number of uMC cases (10%) in the present study. Based on abovementioned considerations it is plausible that the majority of the uMC cases identified were either LC cases, or cases not fully meeting the criteria for either CC or LC. When all uMC cases were considered LC cases, this would have resulted in mean annual incidence rates of LC of 1.6 per 100,000 person years in 2000-2012, being still below the lowest incidence rates published so far. Although the overall incidence rates clearly increased, the incidence rate of uMC remained stable over time. This could point towards an improved knowledge of MC and its specific subtypes by both gastroenterologists and pathologists.

Strengths of the present study reside in the fact that the PALGA registry has an almost full national coverage for MC, enabling the establishment of a large population-based cohort of over 16.5 million inhabitants. Furthermore, the broad thirteen-year time window showed possible changes in the mean annual incidence rates over time. The main limitations of the present study were that diagnoses were based upon pathologists' conclusions instead of full pathology reports and lack of information on medical history, clinical symptoms and colonoscopy indications. Biopsy revision of all cases was not possible, considering the high number of cases and the fact that the evaluated excerpts came from 55 different pathology sites.

This is the second study presenting nationwide MC incidence rates from a large population covering over 16 million inhabitants. The Dutch population-based incidence

rates of MC were considerably lower than incidence rates published so far. Both CC and LC showed a significant increase in incidence rates over time and a clear predominance for elder females, which is clinically relevant information considering the ageing population. Further research on environmental and life style risk factors may reveal explanations for the discrepancy in international incidence rates and changes over time.

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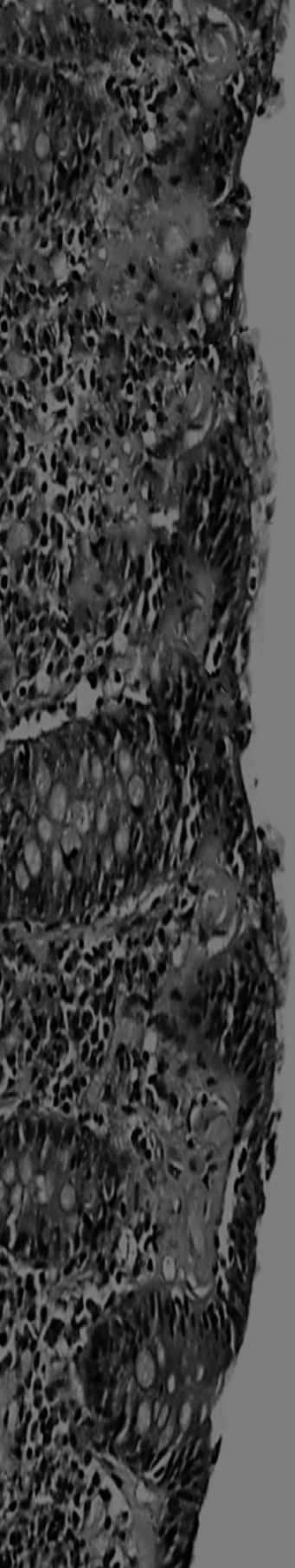

3

Clinical characteristics of microscopic colitis patients in the southeast of the Netherlands: a retrospective cohort study

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To be submitted

Abstract

Introduction

The incidence of MC in the Netherlands is low compared to other countries. Hypothetically, a different clinical presentation might impede patient identification. Therefore, this study aimed to compare clinical characteristics of Dutch MC patients with other populations and to describe the outcomes of the first applied treatment strategies after diagnosis, based on real-life treatment data.

Methods

MC cases, registered in the Dutch nationwide pathology registry (PALGA) between 2000-2012, were identified from all hospitals in the southeast of the Netherlands. Cases were eligible for inclusion when >18 years old, a medical file was available, and the diagnosis was confirmed based on histological and clinical data. Clinical symptoms, risk factors and applied treatment strategies were retrieved from patient's medical files.

Results

In total, 533 cases (77.1% females, median age 61.2 years (IQR 51.1-70.6 years)) were available for analysis. The median follow-up time after diagnosis was 24.4 months [IQR 6.6-57.6 months]. CC patients had more daily stools (p=0.01), a longer duration of symptoms (p<0.01), more often had rheumatoid arthritis (p=0.01) and more often received medical treatment (p=0.05) compared to LC. Budesonide was more effective to induce clinical response compared to other drugs, when applied as first treatment option.

Conclusion

Clinical characteristics of Dutch MC patients were in line with other populations and therefore an unlikely explanation for the low Dutch incidence rates. Furthermore, our data reflect that CC seems to exhibit a more severe disease course than LC and that oral budesonide is more effective than other drugs as first treatment strategy.

Introduction

Microscopic colitis (MC) is an intestinal disorder, hallmarked by chronic, watery, non-bloody diarrhea in combination with a normal macroscopic appearance of the colonic mucosa and typical histological changes in the biopsy samples. MC symptoms significantly influence patient's health related quality of life.[1] On average, MC patients are >60 years old at diagnosis and most often of female gender.[2]

Histologically, MC is classified into two major subtypes, being collagenous colitis (CC) and lymphocytic colitis (LC). CC is characterized by a thickened sub epithelial collagen band of ≥10µm, while an increased number of intraepithelial lymphocytes (>20/100 epithelial cells) is the main feature of LC.[3] Histological changes not completely fulfilling the criteria of either LC or CC are classified as incomplete MC (MCi). In general, the clinical presentation of CC and LC is similar.[4] Watery diarrhea, with an average frequency of 6 times per day is the main symptom. Fecal incontinence (17-59%), nightly defecation (15-59%) and moderate weight loss (26-59%) are also regularly reported.[5-9] If present, abdominal pain is mostly mild. A difference between the two subtypes is the duration of clinical symptoms before diagnosis, which appears to be longer in CC compared to LC patients (16 vs. 4 months, respectively).[4]

The knowledge on the disease course of MC is limited. Data, when present are predominantly derived from retrospective studies. Of what is currently known, the majority of patients has a chronic or relapsing-intermittent disease course[10,11] and a minority of 5-15% experiences spontaneous remission after diagnosis.[7] The clinical characteristics of MC patients in the Netherlands have never been described in detail. The incidence of MC in the Netherlands appears to be strikingly low compared to other European countries.[12] Therefore, it would be interesting to assess whether the clinical presentation is different. Hypothetically, a different clinical presentation might impede proper patient identification based on the classical disease characteristics.

International variation in risk factor exposure might also contribute to varying international incidence rates. Several factors are associated with an increased risk of MC. Especially cigarette smoking has been consistently associated with MC. In addition, current smoking negatively affects MC disease course and advances the age of diagnosis by about 10 years.[13-16] Other factors associated with an increased risk of MC are the presence of concomitant autoimmune disorders (*e.g.* rheumatoid arthritis, thyroiditis), celiac disease and bile acid malabsorption.[17,18] Last, several drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) have been found to be associated with an increased risk of MC.[19,20]

Oral budesonide is the treatment regimen of first choice in MC. However, about 10-20% of patients does not achieve clinical remission after a 6-8 week treatment course with 9mg in clinical trials.[21,22] In the responders, a clinical relapse occurs in 60-80% of cases after discontinuation of treatment.[23,24] Retreatment with oral budesonide results in a recurrence of clinical remission in 80-85% of the patients. For the 15-20% that

eventually fail to achieve clinical remission on budesonide, no alternative evidence-based treatment option is available. In literature bile acid binders, mesalazine, thiopurines and even biologicals are reported to be applied, but with varying clinical response rates. Unfortunately, real-life effectiveness data of oral budesonide and other treatment modalities are limited to a handful of cohort studies.[7-10] This impedes to assess true drug effectiveness in the less well-controlled patient population of daily clinical care. In addition, most of these studies, except for one,[7] reported on the general effectiveness of the applied drugs, regardless of the moment the drug was prescribed during treatment. It would be interesting to be more specifically informed on the effect when the drugs are applied as first treatment strategy. In that way, the effectiveness of *e.g.* budesonide could be assessed in a non-controlled population and could be explored whether other drugs than oral budesonide might be effective as primary treatment for MC. Real-life treatment data are also useful to compute the average treatment duration and cumulative dosages of oral budesonide in every day clinic.
Therefore, the primary aim of this study was to describe clinical characteristics of Dutch MC patients, in order to assess whether differences in clinical characteristics could explain for the low national incidence rates. Secondly, the study aimed to report real-life data on the use and effectiveness of oral budesonide as well as other treatment modalities applied as initial treatment for MC.

Methods

Study population

MC cases from all ten hospitals in the southeast of the Netherlands were identified via PALGA, the Dutch nationwide registry of histo- and cytopathology. Inclusion criteria were: a PALGA registered diagnosis of MC, CC or LC in one of the participating clinical centres between January 2000 and December 2012 and an age above 18 years. All pathology reports were re-evaluated and patient's medical charts were reviewed using standardised registration forms. Cases were excluded if no medical charts were available, or when the diagnosis could not be confirmed based on histological and/or clinical data. To assess the reliability of the pathology reports, almost two-third of the biopsy slides was reviewed by an experienced pathologist (D.G.), according to the established international diagnostic criteria for MC.[3] If the diagnosis was not confirmed upon revision, cases were excluded. If no revision was performed, the diagnosis as recorded in the official pathology report was adopted, provided the diagnosis was described as likely / definitely positive. Any cases with a diagnosis of incomplete CC (CCi) or incomplete LC (LCi) were added to the CC and LC group, respectively, for statistical analyses.

Clinical data

Medical charts were reviewed for: age, gender, type of diagnostic endoscopy, location of the diagnostic biopsies, date of first symptoms, clinical symptoms, comorbidities, medication use, smoking status (all at the time of diagnosis). The date of diagnosis was defined as the date of histological diagnosis. Lost-to-follow-up was defined as the date of the last visit to the gastroenterology outpatient clinic or date of death. Any information on the applied treatment strategy was recorded, including drug type, dosage, prescription dates, and the reasons to prescribe, taper, withdraw, restart, switch or stop a treatment. A treatment course was considered continuous until a gap of >30 days between subsequent prescriptions of the same drug was observed, regardless of dosage. Within the budesonide users, response to treatment was assessed at 3, 6, 9, and 12 months after diagnosis and defined as a successful tapering of a continuous treatment course followed by a treatment-free period of at least 30 days. If the first continuous course lasted longer than the predefined time window, or if a different drug had to be initiated within 30 days after tapering, cases were classified as budesonide non-responder. In the responders, the percentage of subjects needing treatment for a relapse of symptoms >30 days after successful tapering were recorded.

Statistical analysis

For continuous variables, medians with interquartile ranges (IQR) were calculated. Differences between groups were calculated using a Mann-Whitney-U or Chi^2-test. Statistical significance was determined as $p<0.05$. Analyses were conducted with IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Population

A total of 819 cases with a registered diagnosis of MC within the designated catchment area and time period, were identified from the PALGA registry. A subgroup of 289 cases (34.9%) was excluded because: patient files were not available (n=116) (*e.g.* when colonoscopies were requested by GPs), the diagnosis was established before the year 2000 (n=8), the diagnosis could not be confirmed based on the pathology report (n=49), the histological diagnosis was not acknowledged as MC by the clinician based on clinical data (n=101), or the initial histological diagnosis was not confirmed after biopsy revision (n=12). This resulted in a study population of 533 MC cases, of which 411 females (77.1%) (Table 3.1). The median age at diagnosis was 61.2 years (IQR 51.1-70.6 years). The median follow-up time after diagnosis was 24.6 months [IQR 8.1-62.1 months] and 23.7 months [IQR 5.9-52.0] for CC and LC cases, respectively (p=0.17).

Table 3.1 Clinical characteristics of MC patients in the Netherlands

	MC (n=533)	CC (n=268)*	LC (n=265)^	p-value[#]
Female gender, n (%)	411 (77.1%)	214 (79.9%)	197 (74.3%)	NS
Median age at diagnosis, years (IQR)				
Total population	61.2 (51.1-70.6)	59.9 (49.7-70.6)	61.4 (51.0-70.9)	NS
Males	62.8 (54.1-62.8)	59.0 (49.0-67.0)	64.4 (57.0-73.7)	0.03
Females	60.7 (50.6-70.5)	60.5 (50.6-71.1)	60.9 (50.7-69.5)	NS
Median duration of symptoms before diagnosis, weeks (IQR)	24 (11-69)	24 (11-75)	17 (9-42)	<0.01
Clinical symptoms at diagnosis				
Diarrhea, n (%)	514/518 (99.2%)	259/260 (99.6%)	255/258 (98.8%)	NS
Median number of daily stools (IQR)	4.5 (3.0-7.0)	5.0 (3.5-7.5)	4.5 (3.0-6.0)	0.01
Nightly diarrhea, n (%)	96/513 (18.7%)	54/257 (21.0%)	42/256 (15.8%)	NS
Fecal incontinence, n (%)	49/511 (9.6%)	31/255 (12.2%)	18/256 (6.8%)	NS
Abdominal pain, n (%)	173/51 (33.8%)	89/257 (34.6%)	84/255 (32.9%)	NS
Bloating, n (%)	34/512 (6.6%)	21/257 (8.2%)	13/255 (5.1%)	NS
Flatulence, n (%)	46/511 (9.0%)	19/257 (7.4%)	27/254 (10.6%)	NS
Weight loss, n (%)	169/513 (32.9%)	87/257 (33.9%)	82/256 (32.0%)	NS
Diagnostics				
Colonoscopy, n (%)	470/533 (88.2%)	228/268 (85.1%)	242/265 (91.3%)	0.03
Treatment (at least 1 prescription during disease course)				
No	58/521 (11.1%)	22/265 (8.3%)	36/256 (13.6%)	0.05
Budesonide	363/522 (68.1%)	191/265 (72.1%)	172/257 (64.9%)	NS
Mesalazine	163/522 (31.2%)	79/265 (29.5%)	84/257 (31.7%)	NS
Prednisone	39/522 (7.5%)	19/265 (7.1%)	20/257 (7.5%)	NS
Loperamide	124/522 (23.3%)	76/265 (28.4%)	48/257 (18.1%)	<0.01
Antibiotic	24/522 (4.8%)	19/268 (7.2%)	5/257 (1.9%)	<0.01
Mebeverine	22/522 (4.1%)	7/265 (2.6%)	15/257 (5.7%)	NS
Cholestyramine	27/522 (5.1%)	19/265 (7.2%)	8/257 (3.0%)	0.05
Fibers	27/522 (5.2%)	13/265 (4.9%)	14 (5.9%)	NS
Any thiopurine	24/522 (4.6%)	15/262 (5.7%)	9/256 (3.5%)	NS
Methotrexate	4/512 (0.8%)	2/261 (0.8%)	2/251 (0.8%)	NS
Biological	7/517 (1.4%)	4/262 (1.5%)	3/255 (1.2%)	NS
Other	8/522 (1.5%)	4/265 (1.5%)	4/257 (1.6%)	NS
Hospitalization for MC	42/508 (8.3%)	23/255 (9.0%)	21/254 (8.3%)	NS
Diagnosis of MC as reason for first hospitalization	19/42 (45.2%)	10/23 (43.5%)	9/19 (47.4%)	NS
>1 hospitalization	5/42 (11.9%)	2/23 (8.7%)	3/19 (15.8%)	NS
Comorbidity at diagnosis[$]				
No comorbidity	70/511(13.7%)	42/260 (16.2%)	42/251 (11.2%)	NS
Malignancy	50/470 (10.6%)	27/268 (10.1%)	23/258 (8.9%)	NS
Arthritis				
Rheumatoid arthritis	27/511 (5.3%)	20/260 (7.7%)	7/251 (2.8%)	0.01
Non-rheumatoid arthritis	16/511 (3.1%)	6/260 (2.3%)	10/251 (4.0%)	NS
Thyroid disorder				
Hypothyroidism	31/511 (6.1%)	15/260 (5.8%)	16/251 (6.4%)	NS
Other thyroid disorder	24/511 (4.7%)	12/260 (4.6%)	12/251 (4.8%)	NS
Diabetes		19/260 (7.3%)	16/251 (6.4%)	
Type I diabetes	4/511 (0.8%)	2/260 (0.8%)	2/251 (0.8%)	NS
Type II diabetes	32/511 (6.3%)	18/260 (6.9%)	14/251 (5.3%)	NS

Table 3.1 (continued)

	MC (n=533)	CC (n=268)*	LC (n=265)^	p-value[#]
Other autoimmune disorder	27/511 (5.5%)	13/260 (5.0%)	14/251 (6.0%)	NS
Cardiovascular	222/511 (43.6%)	111/260 (42.7%)	112/251 (44.6%)	NS
Pulmonic	49/511(9.6%)	18/260 (6.9%)	31/251 (12.4%)	0.04
Gastroenterological	124/511 (24.3%)	59/260 (22.7%)	65/251 (25.9%)	NS
Other	323/511 (63.2%)	157/260 (60.4%)	166/251 (66.1%)	NS
Drug use at diagnosis				
No drug use	74/494 (15.0%)	44/249 (17.7%)	30/245 (12.2%)	NS
PPI	14/494 (2.8%)	5/249 (2.0%)	9/245 (3.7%)	NS
NSAID	19/494 (3.6%)	12/249 (4.8%)	7/245 (2.9%)	NS
SSRI	22 (4.8%)	11/249 (4.4%)	11/245 (4.5%)	NS
Smoking status at diagnosis				
Current smoker	194/389 (49.9%)	103/203 (50.7%)	91/186 (48.9%)	NS
Former smoker	63/389 (16.2%)	27/203 (13.3%)	36/186 (19.4%)	NS
Never smoker	132/389 (33.9%)	73/203 (36.0%)	59/186 (31.7%)	NS

* Including 12 cases of incomplete collagenous colitis; ^ Including 22 cases of incomplete lymphocytic colitis; [#] Collagenous versus lymphocytic colitis; [$] At least one comorbidity prior to diagnosis, within any of the predefined categories. *MC: Microscopic colitis, CC: Collagenous colitis, LC: Lymphocytic colitis; IQR: Inter quartile range*

Based upon either the biopsy revision (342/533 cases, 64.2%) or the pathology report (191/533 cases, 35.8%), 256 patients (48.0%) were diagnosed with CC, 243 (45.6%) with LC and 34 (6.4%) with incomplete MC (MCi). In 80.4% of the cases, the result of the biopsy revision was in line with the original report (Table 3.2). The concordance increased to 87.7%, when incomplete forms (CCi and LCi) were considered as CC or LC. As MCi relatively new histological entity, MCi was never diagnosed by pathologists in any of the included cases and only reported during the process of biopsy revision.

Table 3.2 MC subtype classification based on pathology report vs. biopsy revision

		Biopsy revision				
		CC	LC	CCi	LCi	
Pathology report	CC	129	13	6	3	151
	LC	20	146	4	19	189
		149	159	10	22	342

Clinical data

An overview of the clinical characteristics is provided in Table 3.1. Almost all patients (96.4%) had diarrhoea at diagnosis, with a median number of daily stools of 5 (IQR 3-7). About 18% of patients reported nightly defecation and about 10% faecal incontinence. Abdominal complaints and weight loss were documented in 32.4% and 31.7% of the patients, respectively. In the vast majority of cases, the diagnosis was established by full colonoscopy (84.2%) and random biopsy sampling (87.2%). The median time between

the first complaints and the date of histological diagnosis was significantly longer in CC (24 weeks, IQR 11-75), compared to LC (17 weeks, IQR 9-42) (p<0.01).
The clinical symptoms of LC and CC were similar (Table 3.1). Compared to LC, CC patients had more daily stools (5.0 versus 4.5, p=0.01) and more frequently had rheumatoid arthritis (p=0.01) or a pulmonary disorder (p=0.04) as concomitant disease. The number of CC and LC cases exposed to MC associated drugs (*e.g.* NSAID, PPI), or with ≥1 hospitalization during the disease course was not statistically different (Table 3.1). In nearly half of the patients, the reason for the first hospitalization was to diagnose MC. The median time until the first hospitalization, excluding those to diagnose MC, was 589 days (IQR 126-511 days) and 447 days (IQR 108-767 days) for CC and LC, respectively (p=0.38).
There was no statistical difference between CC and LC with regard to another major MC risk factor, current smoking at diagnosis. At diagnosis, current smokers were about 7 years younger than former and never smokers (54.4 ± 15.9 vs. 61.8 ± 19.9 years; p=0.22), which is known from literature. They also had more bowel movements per day (6.6 ± 4.4 vs. 5.0 ± 3.2; p<0.001).

Treatment

No difference was observed between CC and LC with regard to the first treatment modality applied after diagnosis (Table 3.3). In the majority of cases, the first applied treatment was oral budesonide (46-49%) or oral mesalazine (21-24%). The preference for budesonide as first treatment strategy increased over time, reaching 58% in 2012 (Figure 3.1). Of all MC patients, 11.1% (n=59) received no treatment after diagnosis, which was more common for LC compared to CC (7.8% vs. 14.3%, respectively, p=0.02) (Table 3.3). Considering all treatment modalities ever applied during the disease course, CC patients more often received loperamide (p<0.01), cholestyramine (p=0.05), or an antibiotic (p<0.01), compared to LC patients (Table 3.1).

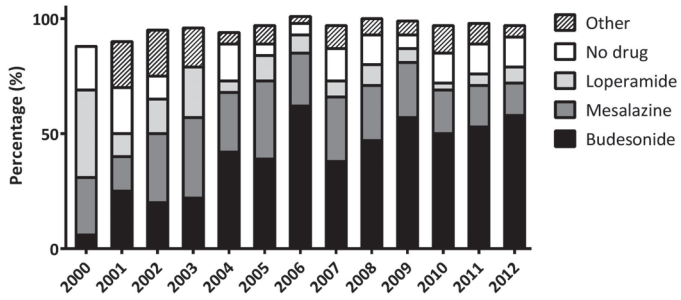

Figure 3.1 Drugs applied as first treatment after diagnosis, per year

Table 3.3 First applied treatment after diagnosis

	MC (n=533)	CC (n=268)*	LC (n=265)^	p-value[#]
No medical treatment	59 (11.1%)	21 (7.8%)	38 (14.3%)	0.02
Budesonide	250 (46.9%)	130 (48.5%)	120 (45.6%)	NS
Mesalazine	120 (22.5%)	57 (21.3%)	63 (23.8%)	NS
Prednison	3 (0.6%)	- (0.0%)	3 (1.1%)	NS
Loperamide	46 (8.6%)	27 (10.1%)	19 (7.2%)	NS
Antibiotic	21 (3.9%)	15 (5.6%)	6 (2.3%)	NS
Mebeverine	5 (0.9%)	3 (1.1%)	2 (0.8%)	NS
Cholestyramine	7 (1.3%)	5 (1.9%)	2 (0.8%)	NS
Fibers	9 (1.7%)	4 (1.5%)	5 (0.2%)	NS
Missing data	13 (2.4%)	6 (2.2%)	7 (2.7%)	NS

* Including 12 cases of incomplete collagenous colitis; ^ Including 22 cases of incomplete lymphocytic colitis; [#] Collagenous versus lymphocytic colitis. MC: Microscopic colitis, CC: Collagenous colitis, LC: Lymphocytic colitis; IQR: Inter quartile range

Almost 70% (n=363) of all patients received at least one prescription of budesonide anytime during follow-up (Table 3.1). In 250 of all patients (46.9%), oral budesonide was given as first treatment modality after diagnosis, of whom 244 had detailed prescription data available. The median duration of the first continuous course of oral budesonide was 200 days (IQR 93-581 days) for CC and 162 (IQR 91-424 days) for LC (p=0.34). The number of responders at 3, 6, 9, or 12 months after diagnosis, that achieved complete tapering of the first continuous course and a follow-up long enough to determine the response, was 95/138 (68.8%), 114/173 (65.9%), 121/183 (66.1%) and 125/189 (66.1%), respectively (Figure 3.2). Of the responders within 3, 6, 9 or 12 months of continuous treatment, 58 (61.1%), 67 (58.8%), 71 (58.7%), and 73 (58.4%) patients received a new course of oral budesonide for relapsing symptoms after a treatment-free period of at least 30 days, respectively. The median time to the second course was 131 days (IQR 64-389 days).

Cumulative dosages of prescribed budesonide could be calculated for 351/363 treated cases. The median cumulative dose of oral budesonide in this population was 2421mg (IQR 866-4875 mg) for CC and 1890mg (IQR 695-3905 mg) for LC patients (p=0.10). After correction for follow-up time, this resulted in a median daily dose of 3.1mg (IQR 1.4-4.9 mg) for CC and 3.2 mg (IQR 1.3-5.1 mg) for LC patients (p=0.99).

Mesalazine was prescribed as first treatment modality in 120/533 (22.5%) of patients (Table 3.4). In 16.7% of them treatment was successfully tapered, in 36.6% there was no or a transient clinical effect. In the end, 47.3% of patients with mesalazine as first treatment strategy switched to oral budesonide during follow-up. This was not different between CC and LC.

Table 3.4 Reasons to stop the first continuously used treatment after diagnosis, regardless of follow-up time

	MC (n=533)	Clinical effect with tapering	No clinical effect	Loss of clinical effect	Side effects	Other reason to stop	Ongoing at end of follow-up	Missing data[#]
No medical treatment	59 (11.1%)							
Budesonide	250 (46.9%)	140 (56.0%)	6 (2.4%)	12 (4.8%)	3 (1.2%)	12 (4.8%)	53 (21.2%)	24 (9.6%)
Mesalazine	120 (22.5%)	20 (16.7%)	4 (3.3%)	40 (33.3%)	8 (6.7%)	12 (10.0%)	18 (15.0%)	18 (15.0%)
Prednisone	3 (0.6%)	1 (33.3%)					1 (33.3%)	1 (33.3%)
Loperamide	46 (8.6%)	5 (10.4%)		7 (15.2%)		1 (2.2%)	10 (21.7%)	22 (47.9%)
Antibiotic	21 (3.9%)	15 (71.4%)	1 (4.8%)	1 (4.8%)	1 (4.8%)	3 (14.3%)		
Mebeverine	5 (0.9%)			2 (40.0%)		1 (20.0%)		2 (40.0%)
Cholestyramine	7 (1.3%)			2 (28.6%)		1 (14.3%)	1 (14.3%)	4 (42.8%)
Fibers	9 (1.7%)	2 (22.2%)		3 (33.3%)		1 (11.0%)	2 (22.2%)	3 (33.3%)
Missing data	13 (2.4%)							

MC: Microscopic colitis, CC: Collagenous colitis, LC: Lymphocytic colitis; IQR: Inter quartile range

Other drugs such as loperamide, mebeverine, cholestyramine, fibres or prednisone, all rarely applied as first treatment after diagnosis, were often stopped (*e.g.* for loss of clinical response) (Table 3.4). Although the exact reason to stop these drugs was often not documented, about 60% received another drug (*e.g.* budesonide or mesalazine) hereafter. Only a small proportion of the total patient population required immunosuppressive treatments such as thiopurines (4.6%), biologicals (1.4%) or methotrexate (0.8%) ever during their disease course (Table 3.1).

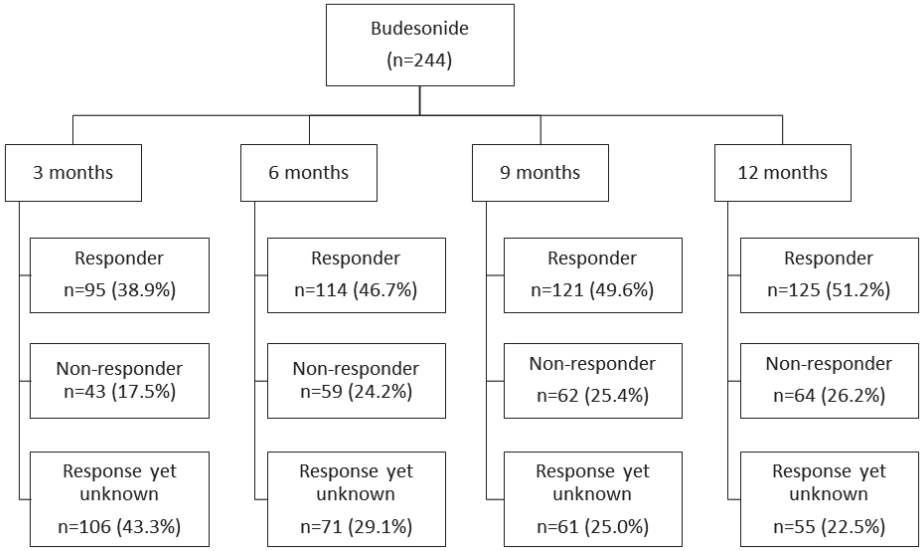

Figure 3.2 Response to the first continuous dose of oral budesonide 3, 6, 9, or 12 months after diagnosis. Response was defined as a complete tapering of the first continuously used prescription of oral budesonide after diagnosis within the defined time window, followed by a treatment free period of at least 30 days.

Discussion

This is the first, detailed description of real-life clinical and treatment data in MC patients and the first report to describe the clinical characteristics of Dutch MC patients. Although the clinical presentation of CC and LC was generally similar, CC can be considered to exhibit a more severe disease course based on the significantly longer duration of symptoms, more daily stools, more frequent need for medical treatment, compared to LC. In addition, we showed that budesonide was the most prescribed and the most effective treatment strategy compared to other drugs, in this population.

Compared to a systematic review, our data on the clinical characteristics of Dutch MC patients showed a similar age and gender distribution.[4] The average number of daily stools was also comparable. However, the proportion of CC and LC patients with abdominal pain or weight loss was considerably lower, being 44-50% in the review compared to 30-35% in the current cohort. Also, the proportion of patients that reported nocturnal stools (16-21%) was low in comparison to other studies.[5,10,11] Although the current data are in line with a recent Swedish cohort study of Mellander *et al.,*[25] underreporting of these clinical features in patient medical charts is likely to contribute to this difference. The significantly longer duration of symptoms before diagnosis in CC was in line with the pooled data of Rasmussen *et al.,*[4] although the difference between CC and LC was less pronounced in the current study. A clear explanation for this finding is lacking and even is unexpected considering the pronounced histological abnormalities in CC (*i.e.* a thickened sub epithelial collagen band).

Thyroid disease, rheumatoid arthritis, diabetes mellitus and celiac disease have frequently been associated with MC. In the present study, the proportion of patients with a thyroid disorder, rheumatoid arthritis or diabetes was comparable to the pooled data of Rasmussen *et al.*[4] and data from a previous study, comprising a small subset of the current population.[26] The significantly smaller proportion of patients with rheumatoid arthritis in the LC compared to the CC population was also observed in this systematic review.[4] Unfortunately, the number of patients with concomitant celiac disease in our population could not be reliably assessed based on the reviewed medical charts. Another strong risk factor for MC, *i.e.* current smoking,[13-16] was present in about half of the study population, a finding that is in line with other studies.[4,8,9]

Remarkably, the prevalence of medication use at time of diagnosis was clearly different compared to literature. The proportion of patients that used NSAIDs or PPIs at diagnosis was only 2-5%. In the studies of Keszthelyi *et al.* and Rasmussen *et al.* this was considerably higher, being 20% and 32-39% for NSAIDs and 37% and 11-28% for PPIs, respectively.[4,26] A likely explanation is the applied definition of drug exposure. In the present study, we specifically assessed the use of concomitant medication at the moment of diagnosis, while other studies often included a defined period (*e.g.* 6 or 12 months) before diagnosis. Underreporting of these (over-the-counter) drugs in medical files has probably contributed to our results as well.

In line with published data, oral budesonide was found to be effective in MC. About two-thirds of the patients responded to their first continuous course of oral budesonide in the first year after diagnosis (Figure 3.2). However, these real-life data also show that in a substantial group of patients (>20%) the first continuous course of oral budesonide was not or could not be tapered after a 12-month treatment period. This reflects that in daily practice, in contrast to clinical trials, clinicians tend to adjust dosages based on the clinical symptoms, slowly tapering the drug, instead of trying a brief (*e.g.* 6-8 week)

induction course followed by cessation of treatment. The lack of clear guidelines on when and how to taper the dose might explain the observed differences between daily practice and clinical trials. The relatively low proportion of patients with a clinical relapse after the first continuous course of budesonide (±60%), compared to literature (±80%), is considered a consequence of the longer duration of the first treatment course.[21,22,27] However, the consequence of longer continuous treatment courses is that patients are generally exposed to high cumulative dosages of corticosteroids.
With 8.4% of patients that stop the first course of budesonide due to no response / loss of response or side effects, oral budesonide performs better than other drugs applied for MC treatment like mesalazine (Table 3.4). Nearly 40% of patients had no (lasting) clinical effect of mesalazine and nearly half of them switched to oral budesonide sometime during the disease course. This finding is in line with results of clinical trials, showing no attributive effect of mesalazine compared to placebo for inducing clinical remission in MC.[28] More than half of the patients received other drugs than budesonide or mesalazine during the disease course (Table 3.1) and about 17% of the patients did not receive one of those two drugs as first treatment after diagnosis (Table 3.3). In this respect, time is a key factor as is visualized in Figure 3.1, clearly showing an increased use of oral budesonide over time. An explanation for the relative infrequent use of oral budesonide as first treatment modality in the first half of the study period (2000-2006) might be the lack of guidelines or systematic reviews on MC treatment and the relative unawareness for the condition among Dutch clinicians, as is reflected by the low incidence rates of MC in the Netherlands in that period.[12]
About 10% of patients, especially LC patients, did not receive any treatment at all. Furthermore, the observed variation in use of loperamide, antibiotics and cholestyramine between CC and LC patients during the disease course should be interpreted with caution. Use of loperamide is not always (well) documented in patient medical files (*i.e.* because of over-the-counter use, GP prescriptions) and antibiotics might be prescribed for a concurrent or suspected parasitic infection. These same arguments should be kept in mind when assessing the effectiveness of these drugs as first treatment strategy.

Strengths of this descriptive study in a real-life population comprise the large number of patients and the ability to define patient eligibility based on both histological (pathology revision or pathology report) and clinical data. Furthermore, the large time window allowed for collection of long-term follow-up data (*i.e.* median follow-up of 24 months). Furthermore, the study population was retrieved from several centers of different setting, including small local, large peripheral hospitals and also one academic hospital. However, some limitations have to be considered. First, the study was based on a retrospective review of data from medical charts. Inevitably, this implies missing data and underreporting of *e.g.* clinical symptoms or drug use. Second, biopsy slides of 191 (35.9%) cases were not revised due to *e.g.* unavailability of slides or quality issues such

as faded staining. Based on the fact that about 20% of patients were diagnosed with a different MC subtype after biopsy revision (Table 3.2), approximately 38 cases included in this study might have been subject to misclassification of MC subtype. We cannot exclude that this might have influence on the study results. Last, data on the applied treatment modalities were based on information from patient files only, instead of pharmacy databases, which may have led to misclassification of exposure.

In summary, this is the first cohort study to report on the clinical characteristics and treatment strategies in the Dutch population. In general, the demographic, clinical and disease characteristics are in line with other populations and are therefore unlikely to explain for the low number of diagnosed patients in the Netherlands. Furthermore, our real-life treatment data reflect that oral budesonide is more effective than other drugs as first treatment strategy for MC. In the future, more presenting long-term, real-life data on the applied treatment strategies and their effects are warranted in order to confirm the treatment

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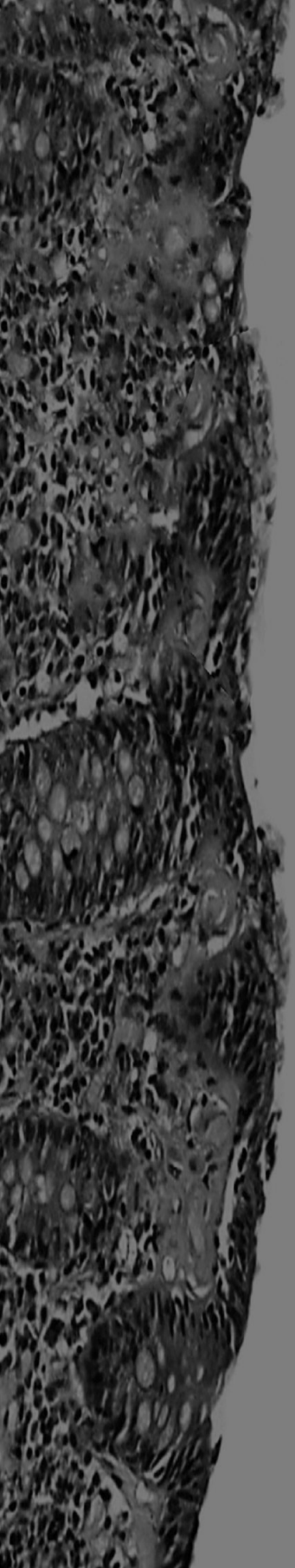

4

High risk of drug-induced microscopic colitis with concomitant use of NSAIDs and proton pump inhibitors: a case-control study

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Abstract

Background

Microscopic colitis (MC) is a chronic bowel disorder characterised by watery diarrhoea. Non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), selective serotonin reuptake inhibitors (SSRIs), and statins have been associated with MC. However, underlying mechanisms remain unclear. Therefore, the aim of this was to study the association between exposure to these drugs and MC, with attention to recency, duration, dosage and combined exposure and to test hypotheses on underlying pharmacological mechanisms.

Methods

A case-control study was conducted using the British Clinical Practice Research Datalink. Cases diagnosed with MC (1992-2013) were matched to MC-naive controls on age, sex, and GP-practice. Drug exposure was stratified according to recency, duration, or dosage of exposure. Conditional logistic regression analysis was applied to calculate adjusted Odds Ratios (AORs).

Results

In total, 1,211 cases with MC were matched to 6,041 controls. Mean age was 63.4 years, with 73.2% being female. Current use of NSAIDs (AOR 1.86, 95% CI 1.39-2.49), PPIs (AOR 3.37, 95% CI 2.77-4.09), or SSRIs (AOR 2.03, 95% CI 1.58-2.61) was associated with MC compared to never or past use. Continuous use for 4-12 months further increased the risk of MC. Strongest associations (5-fold increased risk) were observed for recent concomitant use of PPIs and NSAIDs. Statins were not associated with MC.

Conclusion

Current exposure to NSAIDs, PPIs or SSRs, and prolonged use for 4-12 months increased the risk of MC. Concomitant use of NSAIDs and PPIs showed the highest risk of MC. Acid suppression related dysbiosis may contribute to the PPI effect, which may be exacerbated by NSAID related side-effects.

Introduction

Microscopic colitis (MC) is a chronic disorder of the large intestine, characterized by watery, non-bloody diarrhea. MC is used as an umbrella term for lymphocytic colitis (LC), collagenous colitis (CC), and incomplete MC (MCi). Strict histological criteria are applied to diagnose these subtypes.[1] MC is diagnosed in 10-30% of cases presenting with chronic diarrhea[2] and recent epidemiological studies have reported increased incidence rates over the last decade.[3,4] Treatment with oral budesonide is successful in 81% of cases.[5] However, after cessation of treatment a relapse of symptoms occurs in over 60% of patients, often implying a chronic, intermittent disease course.[6] This contributes to the high impact of MC on patient's quality of life.[7] In order to enable improvement of treatment strategies in future, more insight in the etiology of MC is warranted.

The exact cause of MC is still to be elucidated, although positive associations with auto-immune diseases, bile acid malabsorption, and smoking have been reported. There is increasing evidence that exposure to various drug classes is associated with MC. Studies have reported an elevated risk of MC with use of non-steroidal anti-inflammatory drugs (NSAIDs), proton-pump inhibitors (PPIs), selective serotonin re-uptake inhibitors (SSRIs), and statins.[8-12] Only one study addressed dosage and recency of drug exposure, and generally found stronger associations with current use of NSAIDs or PPIs than with past use.[12] The observed association between NSAIDs and MC appeared to be dose-dependent. Furthermore, NSAIDs and PPIs are often used concomitantly. To date, no studies addressed the effect of duration of continuous use or concomitant use of MC associated drugs on the risk of MC. Such detailed analyses are relevant in order to explore why only a minority of users of the abovementioned drug classes develops MC. Moreover, these data could aid to identify subjects at risk of MC and could provide insight in possible pharmacological mechanisms underlying MC. Although previous observational research speculated on underlying mechanisms,[10-13] no analyses were performed to more specifically test these hypotheses. Therefore, the aim of this study was to evaluate the association between NSAID, PPI, SSRI, and statin use and MC, with attention to the effect of recency of use, duration of continuous use, average daily dose and concomitant use. In addition, the data will be used to indirectly test possible pharmacological mechanisms underlying medication-induced MC.

Materials and methods

Source population

The British Clinical Practice Research Datalink (CPRD) is a longitudinal research database containing active computerized medical records from over 4.4 million inhabitants from 674 primary health care practices in the United Kingdom (UK). The CPRD represents about 7% of the UK population.[14] Data recorded by general practitioners include drug

prescriptions, clinical data, and information on demographics, lifestyle parameters, medical history, laboratory results, and treatment outcomes. Use of the CPRD as a reliable data source has been well validated.[15,16]

Study population

A case-control study was performed using the CPRD. All patients aged 18 years or older, with a record for MC (undefined), CC, or LC between January 01, 1992 and December 31, 2013 were selected (CPRD Medical Codes 30678, 39119 and 35424). The index date of the cases was defined as the date of the first record of MC, CC, or LC. Each case was matched with up to five MC naive controls by year of birth, gender, and GP-practice, using the incidence density sampling technique.[17] Controls were assigned the same index date as their matched case. A minimum period of 12 months of valid data collection prior to the index date was required for each subject upon inclusion. MC cases and their matched controls were excluded in case of a pre-diagnostic colectomy, or a history of inflammatory bowel disease (IBD) or gastro-intestinal cancer.

Exposure

To study the association between the recency of use and the risk of MC, patients were categorized into current, recent, past, or never users of PPIs, SSRIs, NSAIDs, or statins based on the date of the last prescription before index date. A latency period (lag time) of 60 days was taken into account. This was considered the minimal period required for an established diagnosis of MC. All prescriptions and events within the latency period were ignored in order to reduce reverse causation. Current, recent, and past users received their last dispensing 61-90, 91-150, and >150 days before the index date, respectively.

For the main analyses, subjects were stratified according to recency of exposure. These analyses were performed for the whole study population and for CC and LC separately. Subsequently, the duration of continuous use and the average daily dose were calculated for current users. The duration of continuous use was based on the prescribed drug supply and prescribed daily dose. In case of an overlap between two dispensings, or a repeated dispensing within 30 days after discontinuation of the previous period, the duration of continuous use was extended with the time of the last dispensing. In case of any missing data on prescribed drug supply or daily dose, medians were applied. Duration of continuous use was classified into ≤3, 4-12, 13-24, and >24 months of continuous use. The average daily dose was calculated by dividing the cumulative dose by the total treatment time, applying WHO defined daily doses (DDD),[18] and was classified as <0.75, 0.75-1.25, and >1.25 DDDs.

Consistent with other studies,[9,12] crude analyses were adjusted for relevant covariates, *i.e.* in the NSAID group: presence of auto-immune arthritis, irritable bowel syndrome (IBS), PPI use, and SSRI use; in the PPI group: presence of auto-immune arthritis, IBS,

NSAID use, and SSRI use; in the SSRI group: presence of IBD, NSAID use, and PPI use; and in the statin group: smoking status. Here, PPI, SSRI, and NSAID use was defined as any exposure to these drugs in the 6 months prior to index date. A variable was considered a confounding factor when an independent relationship between this variable and both the outcome (MC) as well as the exposure (drug class) was expected.

Statistical analysis

Conditional logistic regression analysis was performed in order to estimate associations between drug exposure and MC (SAS version 9.3, PHREG procedure; SAS Inc. Cary, NC, USA). Adjusted odds ratios (AOR) for MC were estimated by comparing current, recent, or past use of a drug class with never use. Analyses were stratified for recency of use (*i.e.* current, recent, or past use). All analyses were statistically adjusted for potential confounders (*e.g.* concomitant drug use in the 6 months before index date).
A sensitivity analysis was performed by extending the lag time to 90 days, to investigate data robustness. In addition, smoothing spline regression plots were drafted to visualize the association between duration of continuous use and MC.[19]

Additional analyses were performed to test hypotheses on pharmacological mechanisms that might underlie drug-induced MC. Amongst others, the association between MC and concomitant use of NSAIDs and PPIs was studied, as well as single NSAID or PPI use without any co-exposure. Furthermore, the NSAIDs were analysed as total group, as well as for the subgroup of cyclooxygenase (COX) 2 selective NSAIDs separately. Inhibition of the arachidonic acid pathway, leading to an impaired mucosal barrier defence, might be a mechanism involved in the pathophysiology of MC.[11] Separate analyses for individual PPIs were performed in order to test for a drug specific effect, considering a possible association between MC and lansoprazole, specifically.[20] Because acid suppression is assumed a key mechanism in the pathogenesis of PPI induced MC,[13] analyses were performed to test the association between exposure to histamine-2 receptor antagonists (H2RA) and MC. All additional analyses were stratified by recency of use (*i.e.* current, recent, or past use) and exposure definitions were similar as those of the primary analyses.

Ethical approval

The study protocol was approved by the Independent Scientific Advisory Committee for MHRA Database Research (protocol 14_059R2A). The approved protocol was made available to the reviewers of this journal.

Results

Population characteristics

A total number of 1,323 cases with a first diagnosis of MC (undefined), CC, or LC between 1992 and 2013 were selected. Of those, 112 patients fulfilled one or more exclusion criteria (prior colectomy: n=12, IBD: n=94; gastrointestinal malignancy: n=11). The remaining 1,211 cases consisted of 394 CC (32.5%), 292 LC (24.1%), and 525 (43.4%) unspecified MC cases. In total, 6.041 case-matched controls were included. The average time of valid data collection before the index date was 10.3 ± 5.6 years. Further subject characteristics are listed in Table 4.1.

Table 4.1 Baseline characteristics of cases and controls

	Cases		Controls		Crude
	n=1,211	%	n=6,041	%	OR (95% CI)
Female	886	73.2	4,423	73.2	1.00
Mean age at diagnosis (SD)	63.3	14.1	63.2	14.1	1.00
No drug use (6 months before index date)	429	35.4	3,408	56.4	0.38 (0.33-0.44)**
Drug use (6 months before index date)					
NSAIDs	250	20.6	679	11.2	2.09 (1.78-2.46)**
PPIs	506	41.8	1,054	17.5	3.79 (3.29-4.37)**
SSRIs	186	15.4	451	7.5	2.27 (1.88-2.72)**
Statins	327	27.0	1,431	23.7	1.23 (1.06-1.43)*
H2RAs	56	4.6	119	2.0	2.40 (1.73-3.31)**
Presence of (before index date)					
Autoimmune related arthritis	37	3.1	135	2.2	1.37 (0.95-2.00)
Celiac Disease	37	3.1	15	0.2	9.00 (4.79-16.92)*
Irritable Bowel Syndrome	255	21.1	458	7.6	3.36 (2.83-3.99)**
Smoking status					
Never	400	33.0	2,392	39.5	0.74 (0.64-0.84)**
Current	259	21.4	962	15.9	1.47 (1.26-1.73)**
Former	547	45.2	2,541	42.1	1.15 (1.01-1.31)*
Unknown	5	0.4	146	2.4	0.16 (0.07-0.40)**

OR: odds ratio, CI: confidence interval, NSAIDs non-steroidal anti-inflammatory drug, PPIs: proton pump inhibitor, SSRIs selective serotonin reuptake inhibitor, H2RAs:Histamine-2 receptor antagonist. * p<0.05 ** p<0.01

Risk of MC stratified to recency of use

Table 4.2 shows that current use of NSAIDs (AOR 1.86, 95% CI 1.39-2.49), PPIs (AOR 3.37, 95% CI 2.77-4.09), or SSRIs (AOR 2.03, 95% CI 1.58-2.61) was significantly associated with MC when compared to never use. No association was found with current statin use (AOR 1.13, 95% CI 0.94-1.36). Stratification to MC subtypes, showed that current use of PPIs (AOR 5.35, 95% CI 3.79-7.54) and NSAIDs (AOR 2.32, 95% CI 1.46-3.68) was significantly associated with CC, whereas current use of PPIs (AOR 2.06,

95% CI 1.36-3.13) or SSRIs (AOR 2.28, 95% 1.43-3.63) was associated with LC (Supplementary Table S4.1).

Table 4.2 Use of NSAIDs, PPIs, SSRIs, or statins, and the risk of MC, by average daily dose

	Cases		Controls		Crude	Adjusted[a]
	n=1,211	%	N=6,041	%	OR (95% CI)	OR (95% CI)
NSAID use before index date						
Never	308	25.4	2,264	37.5	1.00	1.00
Past use	716	59.1	3,297	54.5	1.65 (1.42-1.91)**	1.39 (1.19-1.62)**
Recent use	94	7.8	229	3.8	3.10 (2.36-4.06)**	2.09 (1.56-2.80)**
Current use	93	7.7	251	4.2	2.84 (2.16-3.73)**	1.86 (1.39-2.49)**
By average daily dose						
One prescription only	4	4.3	10	4.0	3.17 (0.96-10.48)	1.59 (0.43-5.79)
Low (<0.75 DDDs)	62	66.7	177	70.5	2.67 (1.95-3.67)**	1.78 (1.27-2.49)**
Medium (0.75-1.25 DDDs)	21	22.6	45	17.9	3.62 (2.11-6.20)**	2.30 (1.30-4.07)**
High (>1.25 DDDs)	6	6.5	19	7.6	2.46 (0.97-6.21)	1.67 (0.61-4.55)
PPI use before index date						
Never	476	39.3	3,945	65.3	1.00	1.00
Past use	304	25.1	1,175	19.5	2.32 (1.97-2.73)**	1.97 (1.67-2.34)**
Recent use	172	14.2	328	5.4	4.95 (3.98-6.16)**	4.00 (3.19-5.02)**
Current use	259	21.4	593	9.8	4.19 (3.47-5.05)**	3.37 (2.77-4.09)**
By average daily dose						
One prescription only	9	3.5	17	2.9	4.69 (2.04-10.74)*	4.09 (1.75-9.53)**
Low (<0.75 DDDs)	179	69.1	444	74.9	3.85 (3.13-4.75)**	3.05 (2.45-3.79)**
Medium (0.75-1.25 DDDs)	51	19.7	104	17.5	4.85 (3.39-6.96)**	3.90 (2.69-5.66)**
High (>1.25 DDDs)	20	7.7	28	4.7	7.22 (4.01-13.01)**	6.58 (3.61-11.99)**
SSRI use before index date						
Never	821	67.8	4,729	78.3	1.00	1.00
Past use	221	18.2	918	15.2	1.43 (1.21-1.70)**	1.10 (0.92-1.32)
Recent use	48	4.0	150	2.5	1.87 (1.34-2.62)**	1.39 (0.97-1.98)
Current use	121	10.0	244	4.0	2.91 (2.31-3.68)**	2.03 (1.58-2.61)**
By average daily dose						
One prescription only	2	1.7	8	3.3	1.42 (0.30-6.72)	1.02 (0.21-4.97)
Low (<0.75 DDDs)	53	43.8	98	40.2	3.25 (2.30-4.59)**	2.25 (1.55-3.28)**
Medium (0.75-1.25 DDDs)	37	30.6	99	40.6	2.15 (1.46-3.16)**	1.36 (0.90-2.05)
High (>1.25 DDDs)	29	24.0	39	16.0	4.53 (2.76-7.44)**	3.89 (2.26-6.68)**
Statin use before index date						
Never	824	68.0	4,356	72.1	1.00	1.00
Past use	82	6.8	315	5.2	1.43 (1.10-1.86)**	1.36 (1.05-1.77)*
Recent use	107	8.8	465	7.7	1.27 (1.00-1.61)*	1.20 (0.95-1.52)
Current use	198	16.4	905	15.0	1.21 (1.00-1.45)*	1.13 (0.94-1.36)
By average daily dose						
One prescription only	2	1.0	10	1.1	1.13 (0.25-5.15)	1.10 (0.24-5.07)
Low (<0.75 DDDs)	98	49.5	483	53.4	1.12 (0.88-1.42)	1.04 (0.82-1.33)
Medium (0.75-1.25 DDDs)	73	36.9	325	35.9	1.25 (0.95-1.64)	1.18 (0.89-1.55)
High (>1.25 DDDs)	24	12.6	87	9.6	1.59 (1.01-2.51)*	1.29 (0.93-2.33)

OR: odds ratio; CI: confidence interval; NSAID: non-steroidal anti-inflammatory drug; PPI: proton pump inhibitor; SSRI: selective serotonin reuptake inhibitor; DDD: defined daily dose. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date. [a] Adjusted for [NSAIDs] presence of auto-immune arthritis, irritable bowel syndrome (IBS), PPI use , SSRI use; [PPIs] presence of auto-immune arthritis, IBS, NSAID use, SSRI use; [SSRI] presence of IBS, NSAID use, PPI use; [statins] smoking status. PPI, SSRI and NSAID use was defined as any exposure to these drugs in the 6 months prior to index date. * p<0.05 ** p<0.01

A 2- to 4-fold risk of MC was found with recent use of NSAIDs (AOR 2.09, 95% CI 1.56-2.80) or PPIs (AOR 4.00, 95% CI 3.19-5.02) when compared to never use. These associations were not statistically different from current use. After discontinuation of NSAID and PPI use for more than 3 months, the risk of MC dropped to baseline levels (Table 4.2).

Risk of MC and use of PPIs

Current users were stratified for average daily dose and duration of continuous use. Continuous exposure for 4-12 months was associated with the highest risk of MC (AOR 4.69, 95% CI 3.58-6.13). As visualized in Figure 4.1 and Supplementary Table S4.2, the risk of MC decreased after more than one year of continuous use. Although a dose-related effect appeared to exist, differences between dosages were not statistically significant (Table 4.2). When any concomitant NSAID use was excluded, a 2-3 fold significantly increased risk of MC was still observed for PPI use alone (Table 4.3).

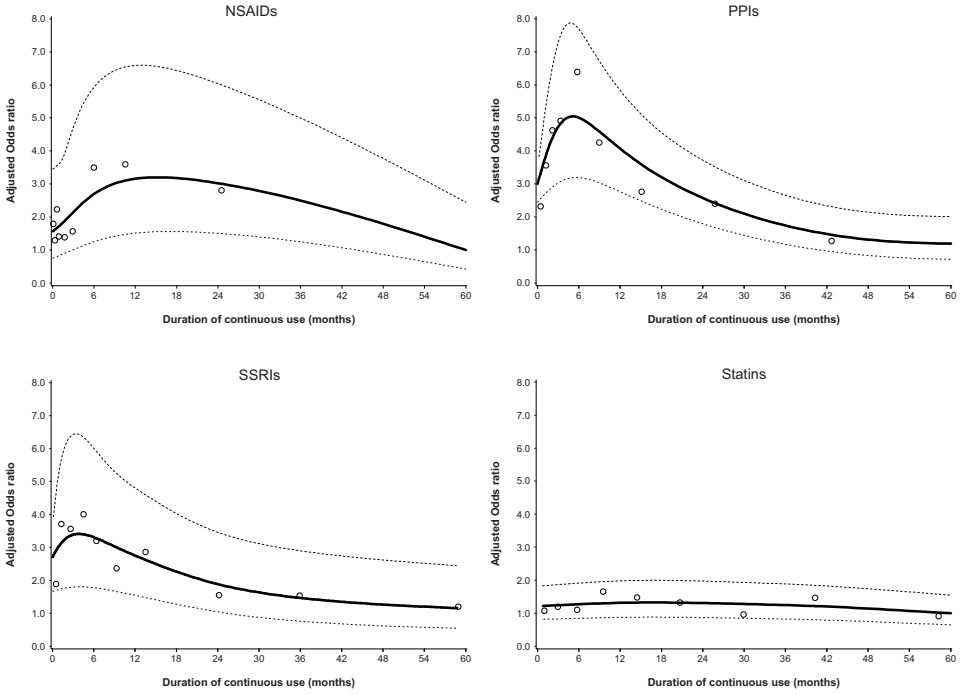

Figure 4.1 Risk of microscopic colitis and duration of continuous use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), selective serotonin reuptake inhibitors (SSRIs) or statins. Solid lines Adjusted Odds ratio, dashed lines 95% confidence bands. Adjusted for the same confounders as listed under Table 4.2

Separate analyses for individual PPIs were performed to test for a specific drug effect, rather than a class effect. Supplementary Table S4.3 shows that beside omeprazole, especially current and recent use of lansoprazole was associated with an increased risk of MC. No or weak associations were found for esomeprazole, pantoprazole, or rabeprazole.

To test the hypothesis of an acid suppression related etiology, a regression analysis on H2RA exposure was performed. Results showed a statistically significant risk of MC in recent and past users of H2RAs, compared to never use (Supplementary Table S4.3).

Table 4.3 Use of NSAIDs or PPIs alone or concomitant use and the risk of MC

	Cases		Controls		Adjusted[a]
	(n=)	%	(n=)	%	OR (95% CI)
NSAID use alone					
Never	124	10.2	492	8.1	1.00
Past use	500	41.3	2497	41.3	0.91 (0.80-1.06)
Recent use	50	4.1	189	3.1	1.31 (0.93-1.86)
Current use	44	3.6	177	2.9	1.29 (0.90-1.86)
PPI use alone					
Never	292	24.1	2173	36.0	1.00
Past use	88	7.3	375	6.2	1.15 (0.89-1.48)
Recent use	128	10.6	288	4.8	2.73 (2.15-3.46)**
Current use	210	17.3	519	8.6	2.41 (1.98-2.92)**
Concomitant NSAID and PPI use					
Never	184	15.2	1772	29.3	1.00
Past use	216	17.8	800	13.2	1.42 (1.19-1.69)**
Recent use	44	3.6	40	0.7	5.40 (3.46-8.42)**
Current use	49	4.1	74	1.2	3.61 (2.46-5.29)**

OR: odds ratio; CI: confidence interval; NSAID: non-steroidal anti-inflammatory drug; PPI: proton pump inhibitor. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date. [a] Adjusted for [NSAID use alone] presence of auto-immune arthritis, irritable bowel syndrome (IBS), PPI use, selective serotonin reuptake inhibitor (SSRI) use; [PPI use alone] presence of auto-immune arthritis, IBS, NSAID use and SSRI use; [Concomitant NSAID and PPI use] presence of auto-immune arthritis, IBS, SSRI use. PPI, SSRI and NSAID use was defined as any exposure to these drugs in the 6 months prior to index date. * p<0.05 ** p<0.01

Risk of MC and use of NSAIDs

Current users were stratified for average daily dose and duration of continuous use. Continuous exposure for 4-12 months yielded the highest AOR in current NSAID users, *i.e.* 3.86 (95% CI 2.28-6.50) and prolonged exposure attenuated this association towards baseline (Figure 4.1, Supplementary Table S4.2). Although a medium average daily dose (0.75-1.25 DDD) showed the strongest association with MC, this was not statistically different from low or high daily doses (Table 4.2).

To test the hypothesis that COX inhibition might be a mechanism leading to mucosal barrier dysfunction, NSAIDs were divided into selective COX-2 inhibitors and other

NSAIDs. However, the associations between MC and these two groups were not statistically different from each other (Supplementary Table S4.3).
When any concomitant PPI use was excluded in the total group of NSAID users, a 30% increased, but statistically non-significant risk of MC was found for recent and current NSAID use (Table 4.3).

Concomitant use of NSAIDs and PPIs

NSAIDs and PPIs are frequently prescribed in combination. Table 4.3 shows that current concomitant use of both NSAIDs and PPIs yielded a higher risk of MC (AOR 3.61, 95% CI 2.46-5.29) than current use of NSAIDs (AOR 1.29, 95% CI 0.90-1.86) or PPIs (AOR 2.41, 95% CI 1.98-2.92) alone. This was also the case for recent concomitant use (AOR 5.40, 95% CI 3.46-8.42).

Risk of MC and use of SSRIs or statins

In current SSRI users, 4-12 months of continuous exposure was associated with the highest risk of MC (AOR 2.68, 95% CI 1.83-3.83) (Figure 4.1 and Supplementary Table S4.2). Dose-dependency was not observed. No associations were observed between statin use and MC.

Sensitivity analysis

In a sensitivity analysis, the latency period was extended from 60 to 90 days. Associations between MC and current use of NSAIDs, PPIs, or SSRIs were stronger than those for recent and past use, when compared to the primary analyses (Supplementary Table S4.4). This was also the case for the concomitant and single use analyses from Table 4.3. However, in all cases current use was again not statistically different from recent use. No significant changes were observed with regard to the average daily dose and duration of use analyses. The sensitivity analysis results did not change the main findings of this study.

Discussion

The results of this case-control study showed that current and recent use of NSAIDs and PPIs were associated with an increased risk of MC, when compared to never and past use, especially in case of continuous exposure for 4-12 months.
However, concomitant use of NSAIDs and PPIs was associated with the highest risks of MC, whereas the associations between NSAIDs or PPIs and MC weakened when any co-exposure to the other drug class was excluded. Only the association between MC and PPI use only remained significant. A positive association was also found with current, but

not recent, SSRI exposure. No statistically significant associations were observed with statin use.

Proton pump inhibitors and MC

The finding that PPI exposure yielded the highest risk of MC, compared to other drug classes, was in line with other observational studies.[9,12,21] Especially current and recent exposure were associated with MC, which was reported by one other study.[12] The observed association remained present after exclusion of any concomitant NSAID use (Table 4.3).

Although it has been established that exposure to PPIs could lead to colonic intra-epithelial lymphocytosis,[22] the exact pathophysiological mechanism of PPI-induced MC is yet unrevealed. Several hypotheses on the underlying mechanisms have been postulated. One of them is an idiosyncratic drug reaction.[13] However, the observed association with recent use and dosage in our study do not support this, as a rapid and dose-independent onset of symptoms would then be expected.

Acid suppression related colonic dysbiosis could contribute to impaired intestinal barrier function and is another hypothesized mechanism on PPI induced MC. Imhann *et al.* recently reported that any exposure to PPIs could induce intestinal dysbiosis in humans.[23] A dysbiosis with clinical implications will take some time to develop. Our finding that 4-12 month continuous exposure to PPIs was significantly associated with MC might support this hypothesis. Because restitution of the normal microbiota composition is expected after discontinuation of exposure, the observed decline in AOR between current and past use is supportive as well. Furthermore, stronger acid suppression is expected to result in a more pronounced alteration in the intestinal microbiota and thus an increased risk of MC. The observed trend towards a dose-dependent effect for PPIs supports this, as does the statistically significant, albeit less pronounced association with the less potent H2RAs.

A variant of the gastric H^+/K^+-ATPase is reported to be present in colonic tissue.[24,25] Inhibition of this pump by PPIs is also suggested as potential mechanism of PPI induced MC.[13] In theory, inhibition of this colonic proton pump might lead to an electrolyte disbalance, altering colonic barrier function. However, binding of PPIs to this colonic proton pump and their subsequent activation is unlikely to play a major role *in vivo.* PPIs are pharmacologically designed to be rapidly absorbed in the upper gastrointestinal tract and targeted to be activated in the highly acidic environment of the gastric canaliculus.[26]

A remarkable finding of this study was the strong association between lansoprazole exposure and MC. It is tempting to assume lansoprazole specifically to be related to MC, considering the number of case-series on MC related to use of lansoprazole.[20] An explanation could be sought in the specific binding of lansoprazole to the cysteine 321 residue of the proton pump.[27] However, it has never been elucidated how this specific binding could lead to an impaired barrier function and/or MC. In this study 95-98% of all subjects were never exposed to pantoprazole, esomeprazole, or rabeprazole. But

despite underpowered calculations, recent use of these PPIs was statistically significantly associated with MC (Table 4.3). A drug-class effect instead of a drug specific effect can therefore not be excluded.

Non-steroidal anti-inflammatory drugs and MC

In the main analysis (Table 4.2), statistical correction for PPI co-exposure in the last 6 months before index date was applied, showing a positive association between current and recent NSAID use and MC. This was consistent with other case-control studies.[9,10,12,21,28-31] However, the statistically significant association between NSAID use and MC was not observed when any concomitant PPI use was excluded. Therefore, our results suggest that the association between NSAIDs and MC as reported in the main analysis of this and other studies, is likely to be based on residual co-exposure to PPIs. Nevertheless, in PPI-users co-exposure to NSAIDs did strengthen the association with MC (Table 4.3).

Animal models have shown that concomitant use of PPIs in NSAID exposed rats significantly aggravated intestinal damage.[32] Pharmacologically, a dysbiosis due to PPI induced gastric acid suppression, in combination with NSAID related effects, could be the underlying mechanism. Their effect on colonic mucosal barrier function by COX inhibition might be attributive herein. The impaired prostaglandin synthesis can increase gut permeability, enhancing the chance for luminal toxins and bacteria to translocate,[30] especially in case of an altered microbiota composition due to gastric acid suppression. As COX-2 is not present in colonic epithelial cells under normal circumstances,[30] exposure to selective COX-2 inhibitors was expected to yield a lower risk of MC, when compared to nonselective NSAIDs. This could however not be confirmed by our results (Supplementary Table S4.3), probably due to insufficient statistical power. Therefore, this hypothesis needs further study.

Another explanation for the reported associations, might be sought in the interaction of NSAIDs with bile. NSAIDs are able to increase bile salt cytotoxicity.[33] Animal models in which bile duct ligation was performed, showed no intraluminal NSAIDs and no signs of gastrointestinal toxicity.[34]

SSRIs and statins and MC

An association was also found between current SSRI use and MC. In line with other studies, this was mainly explained by the association with LC.[9,10]

Serotonin is a relevant substance for gastrointestinal motility, secretion, and perception. Increased 5-HT levels are found in diarrhea predominant IBS.[35] Serotonin is also found to exhibit pro-inflammatory effects in colitis.[36] In MC patients an increased density of serotonin producing cells has been reported and increased serotonin concentrations have been found.[37,38] Findings from a study on serotonin transporter gene polymorphisms were contradictive, indicating these polymorphisms to be but one factor

contributing to the higher serotonin levels in MC.[38,39] Administration of SSRIs increases serotonin levels.[40,41] It might therefore lead to luxation or aggravation of colitis symptoms by interference with the gastrointestinal motility and secretion. However, the relation between SSRI exposure and colonic inflammation remains a black box.
According to the results of the present study, statin use is not associated with an increased risk of MC. Weak associations with MC have been reported, but these studies had methodological shortcomings regarding *e.g.* exposure definition and confounder correction.[9,10] The scarce literature on statins and colitis remains disputable about their presumed anti-inflammatory effect.[42,43]

Cause or bias?

There is a lack of large prospective longitudinal studies that prove causal relationships on drug-induced MC.[44] It is therefore tempting to assume that the general phenomenon of drug-induced MC is based on false associations due to methodological biases or confounding by indication. After all, drugs found to be most associated with MC are prescribed for unspecific abdominal complaints as well. However in MC, abdominal pain is present in only 25-40% of the patients and often mild of nature.[45,46] It is therefore unlikely that PPIs, NSAIDs, the combination of both, or SSRIs will be frequently prescribed for these complaints, and if so, the prescription period will be short. In contrast, 50-70% of the current users in the present study were continuously exposed for more than 3 months.
We acknowledge that PPI with or without NSAID, and SSRI exposure is associated with an increased risk of diarrhea, especially in the elderly,[47] which could lead to a diagnostic bias and reverse causation. However, the incidence of diarrhea due to drug use is about 1% for NSAIDs[48] and 2-4% for PPIs,[49] and often mild, self-limiting, and dose independent.[50] Moreover, studies with the ability to correct for confounding by indication, still reported on an increased risk of MC in NSAID and PPI users.[9,12] Therefore, drug exposure is likely to play a causative role in the development of MC in a selection of patients.

Strengths and limitations

Some limitations of this study have to be acknowledged. First, this was a retrospective study and reliable data on the onset of symptoms relative to the time of first exposure were not available. This hindered proving any causality between NSAID, PPI, or SSRI exposure and MC. Second, we were unable to match our cases with a group of MC-negative controls with chronic diarrhea. Therefore, the reported associations could be an overestimation of the true associations, due to confounding by indication. Third, information on over-the-counter medication was lacking. However, misclassification of exposure was assumed non-differential, because a random distribution of exposure to non-prescribed drugs in case and control group might be assumed. Fourth, no

information on the histology supporting the MC diagnosis was available. Because MC is a histology-based diagnosis, we assumed that established diagnoses will be recorded by GPs in the CPRD database. Nevertheless, some undiagnosed cases might be missed
Despite these limitations, the strength of this study resides in its data source. The CPRD provides reliable and detailed data on patient characteristics, comorbidities, and first-line and clinical drug prescriptions. Furthermore, our methodology allowed for stratification on recency of use, duration of continuous use, and average daily dose and enabled the performance of additional analyses to gain more insight in potential pathophysiological mechanism of drug-induced MC.

Conclusion

In conclusion, this study showed that current and recent use of NSAIDs and PPIs and current use of SSRIs was associated with MC. Concomitant use of PPIs and NSAIDs however, was associated with the highest risk of MC and turned out to be responsible for the observed association between MC and NSAID use as such. Additional analyses indicated that acid-suppression related dysbiosis may contribute to the increased risk of PPI use. The strong association for concomitant PPI and NSAID use indicated that gastrointestinal effects of NSAIDs might aggravate or luxate PPI-related MC.

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Table S4.1 Use of NSAIDs, PPIs, SSRIs, or statins, and the risk of MC, by average daily dose

	CC Cases N= 394	CC Controls N=1,965	LC Cases N=292	LC Controls N=1,450	CC - Adjusted[a]	LC - Adjusted[a]
	%	%		%	OR (95% CI)	OR (95% CI)
NSAID use before index date						
Never	22.3	35.2	27.4	39.0	1.00	1.00
Past use	56.9	56.8	64.4	52.9	1.31 (0.98-1.75)	1.55 (1.14-2.09)*
Recent use	9.1	3.3	3.8	4.0	3.40 (2.02-5.73)**	0.73 (0.35-1.53)
Current use	11.7	4.7	4.5	4.1	2.32 (1.46-3.68)**	0.97 (0.49-1.93)
PPI use before index date						
Never	27.7	61.8	47.3	65.8	1.00	1.00
Past use	26.9	20.4	24.7	19.6	2.75 (2.03-3.72)**	1.59 (1.14-2.23)**
Recent use	16.8	6.4	12.7	5.6	5.25 (3.56-7.76)**	2.90 (1.81-4.66)**
Current use	28.7	11.4	15.4	9.1	5.35 (3.79-7.54)**	2.06 (1.36-3.13)**
SSRI use before index date						
Never	73.9	77.3	67.1	77.9	1.00	1.00
Past use	15.7	15.7	15.1	14.7	0.79 (0.56-1.11)	0.91 (0.62-1.36)
Recent use	3.3	2.6	5.1	2.8	0.75 (0.38-1.50)	1.76 (0.92-3.38)
Current use	7.1	4.3	12.7	4.6	1.00 (0.61-1.65)	2.28 (1.43-3.63)**
Statin use before index date						
Never	64.0	68.4	69.9	71.9	1.00	1.00
Past use	7.4	6.1	8.2	6.3	1.25 (0.81-1.93)	1.34 (0.82-2.19)
Recent use	11.4	8.5	7.2	7.8	1.48 (1.01-2.17)*	0.84 (0.50-1.41)
Current use	17.3	17.1	14.7	13.9	1.03 (0.75-1.41)	1.03 (0.69-1.55)

OR:odds ratio; CI:confidence interval; NSAID:non-steroidal anti-inflammatory drug; PPI:proton pump inhibitor; SSRI: selective serotonin reuptake inhibitor; CC:collagenous colitis; LC:lymphocytic colitis. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date. [a] Adjusted for [NSAIDs] presence of auto-immune arthritis, irritable bowel syndrome (IBS), PPI use , SSRI use; [PPIs] presence of auto-immune arthritis, IBS, NSAID use, SSRI use; [SSRI] presence of IBS, NSAID use, PPI use; [statins] smoking status. PPI, SSRI and NSAID use was defined as any exposure to these drugs in the 6 months prior to index date. * $p<0.05$ ** $p<0.01$

Table S4.2 Current use of NSAIDs, PPIs, SSRIs, or statins and the risk of MC, by duration of continuous use

	Cases		Controls		Adjusted[a]
	n=1,211	%	n=6,041	%	OR (95% CI)
NSAID use before index date					
Never	308	25.4	2264	37.5	1.00
≤3 months	44	47.3	148	59.0	1.43 (0.97-2.10)
4-12 months	29	31.2	43	17.1	3.86 (2.28-6.50)**
13-24 months	8	8.6	19	7.6	2.41 (0.99-5.85)
>24 months	12	12.9	41	16.3	1.20 (0.60-2.39)
PPI use before index date					
Never	476	39.3	3945	65.3	1.00
≤3 months	81	31.3	173	29.2	3.03 (2.33-3.93)**
4-12 months	94	36.3	134	22.6	4.69 (3.58-6.13)**
13-24 months	38	14.7	90	15.2	2.44 (1.68-3.56)**
>24 months	46	17.7	196	33.0	1.45 (1.06-1.99)*
SSRI use before index date					
Never	821	67.8	4729	78.3	1.00
≤3 months	39	32.2	68	27.9	2.14 (1.44-3.20)**
4-12 months	52	43.0	82	33.6	2.68 (1.88-3.83)**
13-24 months	12	9.9	24	9.8	2.55 (1.40-4.64)**
>24 months	18	14.9	70	28.7	0.86 (0.52-1.42)
Statin use before index date					
Never	824	68.0	4356	72.1	1.00
≤3 months	32	16.0	149	16.6	1.01 (0.70-1.47)
4-12 months	58	29.0	201	22.4	1.37 (1.04-1.81)*
13-24 months	39	19.5	159	17.8	1.41 (1.04-1.93)*
>24 months	71	35.5	387	43.2	0.93 (0.73-1.19)

OR: odds ratio; CI: confidence interval; NSAID: non-steroidal anti-inflammatory drug; PPI: proton pump inhibitor; SSRI: selective serotonin reuptake inhibitor. [a] Adjusted for confounding factors as reported under Supplementary Table S4.1. * p<0.05 ** p<0.01

Table S4.3 Use of selective COX-2 inhibitors, nonselective NSAIDs, individual PPIs, or H2RAs and the risk of MC

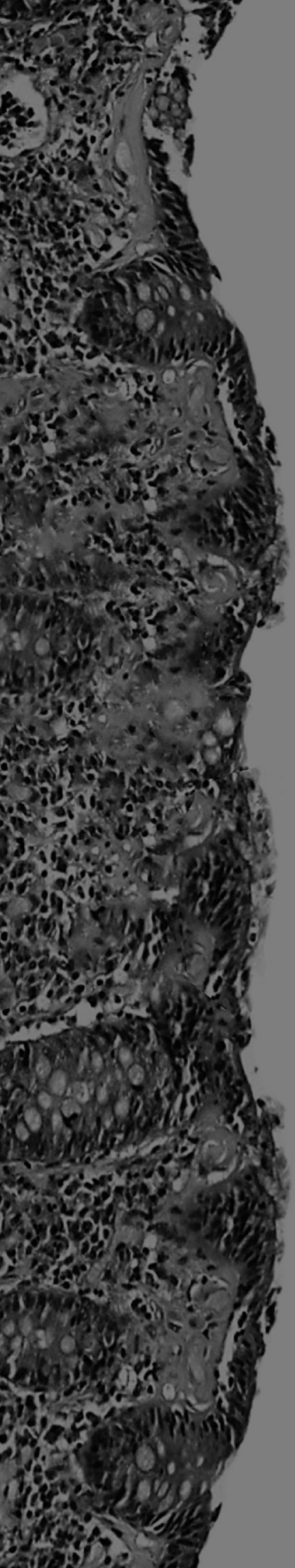
	Cases		Controls		Adjusted[a]
	(n=)	%	(n=)	%	OR (95% CI)
Nonselective NSAIDs					
Never	319	26.3	2347	38.9	1.00
Past use	719	59.4	3247	53.7	1.43 (1.22-1.66)**
Recent use	87	7.2	222	3.7	2.03 (1.51-2.74)**
Current use	86	7.1	225	3.7	1.90 (1.41-2.56)**
Selective COX-2 inhibitors					
Never	1016	83.9	5422	89.8	1.00
Past use	179	14.8	583	9.7	1.21 (0.99-1.48)
Recent use	9	0.7	10	0.2	2.86 (1.05-7.78)*
Current use	7	0.6	26	0.4	1.23 (0.50-3.03)
PPI - Omeprazole					
Never	731	60.4	4626	76.6	1.00
Past use	304	25.1	922	15.3	1.92 (1.63-2.26)**
Recent use	80	6.6	187	3.1	2.26 (1.68-3.03)**
Current use	96	7.9	306	5.1	1.74 (1.34-2.26)**
PPI - Esomeprazole					
Never	1162	95.9	5877	97.3	1.00
Past use	36	3.0	128	2.1	1.10 (0.73-1.65)
Recent use	7	0.6	8	0.1	3.37 (1.17-9.76)*
Current use	6	0.5	28	0.5	1.01 (0.40-2.54)
PPI - Lansoprazole					
Never	700	57.8	4907	81.2	
Past use	263	21.7	778	12.9	2.12 (1.78-2.52)**
Recent use	103	8.5	130	2.2	5.75 (4.28-7.72)**
Current use	145	12.0	226	3.7	4.40 (3.43-5.65)**
PPI - Pantoprazole					
Never	1166	96.3	5923	98.1	1.00
Past use	34	2.8	101	1.7	1.41 (0.91-2.18)
Recent use	6	0.5	6	0.1	3.82 (1.17-12.52)*
Current use	5	0.4	11	0.2	1.71 (0.58-5.05)
PPI - Rabeprazole					
Never	1150	95.0	5858	97.0	
Past use	49	4.0	156	2.6	1.35 (0.94-1.92)
Recent use	4	0.3	4	0.1	5.52 (1.30-23.42)*
Current use	8	0.7	23	0.4	1.48 (0.64-3.45)
H2RA					
Never	923	76.2	5084	84.2	1.00
Past use	247	20.4	856	14.2	1.32 (1.11-1.56)**
Recent use	21	1.7	46	0.8	2.23 (1.31-3.81)**
Current use	20	1.7	55	0.9	1.55 (0.90-2.67)

OR: odds ratio, CI: confidence interval; NSAID: non-steroidal anti-inflammatory drug; COX: cyclooxygenase; PPI: proton pump inhibitor; H2RA: Histamine-2 receptor antagonist. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date. [a] Adjusted for [Unspecific NSAIDs and COX-2 inhibitors] presence of auto-immune arthritis, irritable bowel syndrome (IBS), PPI use , selective serotonin reuptake inhibitor (SSRI) use; [PPIs and H2RAs] presence of auto-immune arthritis, IBS, NSAID use, SSRI use. PPI, SSRI and NSAID use was defined as any exposure to these drugs in the 6 months prior to index date. * p<0.05 ** p<0.01

Table S4.4 Use of NSAIDs, PPIs, SSRIs, or statins and the risk of MC, by average daily dose, with a lag time of 90 days

	Cases		Controls		Crude	Adjusted[a]
	n=1,211	%	n=6,041	%	OR (95% CI)	OR (95% CI)
NSAID use before index date						
Never	303	25.0	2228	36.9	1.00	1.00
Past use	704	58.1	3283	54.3	1.63 (1.41-1.89)**	1.40 (1.20-1.63)**
Recent use	83	6.9	250	4.1	2.61 (1.95-3.48)**	1.76 (1.29-2.39)**
Current use	121	10.0	280	4.6	3.28 (2.54-4.25)**	2.13 (1.61-2.82)**
By average daily dose						
One prescription only	6	5.0	8	2.9	4.16 (1.38-12.51)*	2.89 (0.86-9.68)
Low (<0.75 DDDs)	77	63.6	209	74.6	2.85 (2.10-3.87)**	1.76 (1.26-2.46)**
Medium (0.75-1.25 DDDs)	26	21.5	40	14.3	4.69 (2.77-7.96)**	3.34 (1.89-5.90)**
High (>1.25 DDDs)	12	9.9	23	8.2	4.03 (1.97-8.24)**	3.00 (1.38-6.52)**
PPI use before index date						
Never	485	40.0	3968	65.7	1.00	1.00
Past use	292	24.1	1162	19.2	2.20 (1.87-2.59)**	1.89 (1.59-2.23)**
Recent use	136	11.2	328	5.4	3.84 (3.05-4.83)**	3.14 (2.47-3.98)**
Current use	298	24.6	583	9.7	4.90 (4.08-5.88)**	3.90 (3.23-4.72)**
By average daily dose						
One prescription only	4	1.3	10	1.7	3.52 (1.08-11.45)*	2.60 (0.77-8.71)
Low (<0.75 DDDs)	221	74.2	440	75.5	4.83 (3.96-5.91)**	3.77 (3.06-4.64)**
Medium (0.75-1.25 DDDs)	53	17.8	100	17.2	5.01 (3.52-7.12)**	4.21 (2.93-6.05)**
High (>1.25 DDDs)	20	6.7	33	5.7	5.85 (3.30-10.36)**	5.23 (2.91-9.38)**
SSRI use before index date						
Never	823	68.0	4738	78.4	1.00	1.00
Past use	223	18.4	912	15.1	1.45 (1.22-1.72)**	1.11 (0.93-1.33)
Recent use	57	4.7	144	2.4	2.32 (1.69-3.19)**	1.68 (1.20-2.36)**
Current use	108	8.9	247	4.1	2.57 (2.02-3.27)**	1.89 (1.46-2.46)**
By average daily dose						
One prescription only	3	2.8	3	1.2	5.73 (1.15-28.45)*	5.20 (0.99-27.37)
Low (<0.75 DDDs)	52	48.1	101	40.9	3.03 (2.15-4.28)**	2.23 (1.54-3.25)**
Medium (0.75-1.25 DDDs)	33	30.6	99	40.1	1.96 (1.31-2.93)**	1.26 (0.82-1.95)
High (>1.25 DDDs)	20	18.5	44	17.8	2.65 (1.55-4.51)**	2.45 (1.38-4.36)**
Statin use before index date						
Never	826	68.2	4368	72.3	1.00	1.00
Past use	77	6.4	317	5.2	1.32 (1.01-1.72)*	1.25 (0.96-1.63)
Recent use	108	8.9	460	7.6	1.31 (1.03-1.66)*	1.23 (0.97-1.56)
Current use	200	16.5	896	14.8	1.23 (1.02-1.48)*	1.15 (0.96-1.39)
By average daily dose						
One prescription only	4	2.0	8	0.9	2.73 (0.82-9.07)	2.76 (0.81-9.36)
Low (<0.75 DDDs)	95	47.5	457	51.0	1.12 (0.88-1.44)	1.05 (0.82-1.35)
Medium (0.75-1.25 DDDs)	81	40.5	335	37.4	1.34 (1.03-1.74)*	1.27 (0.97-1.65)
High (>1.25 DDDs)	20	10.0	96	10.7	1.22 (0.75-1.98)	1.10 (0.68-1.80)

OR: odds ratio; CI: confidence interval; NSAID: non-steroidal anti-inflammatory drug; PPI: proton pump inhibitor; SSRI: selective serotonin reuptake inhibitor; DDD: defined daily dose. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date.
[a] Adjusted for confounding factors as reported under Supplementary Table S4.1. * p<0.05 ** p<0.01



5

The relation between NSAID and PPI exposure and microscopic colitis: a combined in vitro and ex vivo approach focusing on barrier function

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Submitted

Abstract

Background

Recently, a strong association between concomitant NSAID/PPI exposure and microscopic colitis (MC) was reported. Although the underlying mechanisms remain unclear, hypotheses predominantly concern (in)direct inhibitory effects of these drugs on the epithelial barrier function in genetically predisposed individuals. The aim of this study was to confirm the association between MC and concomitant NSAID/PPI exposure, and to assess the direct effect of these drugs on colonic epithelial barrier by a combined in vitro and ex vivo approach.

Methods

First, a case-control study was performed in the PHARMO Database Network to confirm recent associations between concomitant NSAID/PPI exposure and MC. Second, CaCo-2 cell monolayers were exposed to NSAIDs, PPIs or both to assess a possible direct effect on the paracellular permeability. Third, fresh colonic biopsies of MC cases in remission and non-MC controls were used in an Ussing chamber experiment, to study the effect of NSAIDs and PPIs on the epithelial barrier ex vivo and to assess host-susceptibility.

Results

Current and recent exposure, and especially concomitant exposure to NSAIDs and PPIs was significantly associated with MC. In vitro, no direct inhibiting effect of the associated drugs on the paracellular permeability was observed. Ex vivo, a direct effect on the epithelial barrier function was not found either. Furthermore, no indications were found for increased host-susceptibility in MC patients.

Conclusion

Although results of observational studies support the association between NSAID and PPI exposure and MC, a direct inhibitory effect of these drugs on the paracellular permeability is unlikely to be a primary underlying mechanism.

Introduction

Microscopic colitis (MC) is a chronic intestinal disorder. The diagnostic hallmarks of the disease are watery diarrhea, a (near to) normal macroscopic appearance of the colon mucosa, and specific histological changes. Based on the histology, two major subtypes of MC can be distinguished, *i.e.* lymphocytic (LC) and collagenous colitis (CC). The exact etiology of MC still remains to be elucidated, but increasing evidence suggests that drug exposure might play a role in MC pathophysiology. Strongest evidence is found for an association between MC and exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs),[1-3] which is most pronounced for CC. Previous data of our group, retrieved from the British CPRD database, showed for the first time that in addition to current and medium-term exposure (4-12 months of continuous use), especially concomitant use of NSAIDs and PPIs was associated with a high risk of MC.[1] Given the international variation in prescription behavior and drug preferences, the reported association between MC and NSAID/PPI co-exposure needs conformation in a separate population. Furthermore, the strong association between MC and NSAID/PPI co-exposure raises questions regarding the underlying mechanisms of drug-induced MC.

NSAIDs are known for their deleterious effects on the gastrointestinal mucosa inducing ulceration, inflammation and bleeding.[4] However, these effects have mainly been reported for the upper gastrointestinal tract. In addition, NSAIDs (*e.g.* indomethacin) increase small intestinal permeability as shown by the urinary excretion of orally ingested sugar probes.[5,6] Data on the effect of NSAIDs on large intestinal permeability are limited and those published showed no effect.[5,7] Nevertheless, NSAIDs are known to induce colon toxicity, which may enhance an effect of a second trigger. NSAID colitis is a well-known, but infrequent manifestation and different from MC in clinical presentation.[8,9]

With regard to PPIs, there is evidence that exposure may lead to perturbations in the colonic microbiota due to reduced gastric acid secretion.[10] It is hypothesized that PPIs might inhibit gastric-like proton pumps present in the distal colon and on certain microbes.[11,12] PPIs were also found to aggravate *Clostridium difficile* induced colitis in mice, which was associated with increased colonic permeability. It is however unclear whether this was a direct effect on the apical junctional complex, regulating paracellular permeability, or an indirect effect via immune activation.[13] An increased permeability may result in permeation of bacteria and their products, resulting in immune activation contributing to drug-induced MC. However, for both PPIs and NSAIDs it is not clear whether they have direct effects on colonic barrier function either alone or as combination.[14-16] As both drugs are rapidly absorbed in the upper GI tract, any effects on the colon are more likely to be related to basolateral instead of luminal drug exposure. Furthermore, it is still unclear why only a small number of patients treated with *e.g.*

NSAIDs and PPIs develop MC. This might indicate that MC patients are genetically more susceptible to drug-induced barrier disruption.
Besides confirming the association of PPI and/or NSAID exposure with MC, this study aimed to look beyond associations and to explore the presence of a possible direct effect of (co-)exposure to NSAIDs and PPIs on paracellular permeability and the role of host-susceptibility herein, using an *in vitro* cell culture model and *ex vivo* Ussing chamber experiment. We hypothesized that NSAIDs and PPIs have a direct inhibitory effect on colonic barrier function, which will be more pronounced in case of concomitant NSAID/PPI administration and in biopsies of MC patients.

Methods

Associations between MC and NSAID / PPI exposure

Source population

First, a case-control study was performed to confirm the reported associations between NSAIDs and PPIs, in a study population with a verified diagnosis. MC cases in the Netherlands were identified in PALGA, the Dutch nationwide registry of histo- and cytopathology, as described previously.[17] All PALGA retrieved MC cases were linked to the PHARMO Database Network. This population-based network combines data from different healthcare settings and comprises more than 4 million residents of a well-defined population in the Netherlands. More detailed information on the set-up of this database can be found elsewhere.[18,19] For this study, the Out-patient Pharmacy Database was used, comprising GP or specialist prescribed healthcare products dispensed by the out-patient pharmacy. Dispensing records include information about type of medicine, dispensing date, strength, dosage regimen and quantity.
All patients with an age of 18 years or older, a PALGA registered diagnosis of CC, LC or unspecified MC between 1 January, 2000 and 31 December, 2012, and a record in the PHARMO Database Network with at least one year of valid data collection prior to the index date were selected. The MC diagnosis was verified based on the pathology report. The index date of the cases was defined as the PALGA registered diagnosis date. Each case was matched with five non-MC controls by year of birth, gender, living area, and follow-up period in the PHARMO Database Network. Controls were assigned the same index date as their matched case.

Distinction was made between exposure to a single drug class (NSAID or PPI) and concomitant NSAID and PPI exposure, based on dispensing dates. In all analyses, patients were classified as current (61-90 days), recent (91-150 days), past (>150 days), or never users based on the date of the last dispensing before the index date. Concomitant

exposure was defined as cases receiving both a NSAID and PPI dispensing in the same period before the index date.
As a diagnostic delay was expected, a lag time of 60 days was taken into account in all analyses. All dispensings and events within this period were neglected. In the single drug class analyses, current users were further stratified according to duration of continuous use and average daily dose. Duration of continuous use was classified into ≤3, 4-12, 13-36, or >36 months of continuous use. Average daily dose was classified as <0.75, 0.75-1.25, >1.25 WHO defined daily dosages (DDD). Further details on the definitions of duration and dosage of use are described in our previous study.[1]
In order to correct for any difference non-concurrent exposure to MC-associated drugs between groups, any exposure to PPIs, NSAIDs, or SSRIs in the 6 months prior to index date was taken into account when assessing association between MC and exposure to NSAIDs, PPIs, or both, respectively.

Statistical analysis

In order to estimate associations between drug exposure and MC, conditional logistic regression analysis was performed (SAS version 9.3, PHREG procedure; SAS Inc. Cary, NC, USA). Adjusted odd ratios were estimated by comparing recency of use (current, recent, past) with never use.

In vitro cell culture experiments

In order to assess a possible direct effect of frequently used PPIs, NSAIDs, or the combination thereof, on the intestinal epithelium, a CaCo-2 cell culture model was used. Cells (passage 35-37) were obtained from the American Type Culture Collection (ATCC, Rockville, USA) and were maintained in Dulbecco's Modified Eagle Medium (DMEM; Lonza Benelux BV, Breda, the Netherlands) containing 4.5 g/L glucose and L-glutamine, 10% fetal bovine serum (Gibco®, Life Technologies Co., Carlsbad, CA, USA), 1% solution of non-essential amino acids (Gibco®), and 1% solution of antibiotic/antimycotic mixture (10,000 units of penicillin, 10,000 mg of Streptomycin, and 25 mg of Amphotericin B per ml; Gibco®) in an atmosphere of 5% CO_2 at 37°C. CaCo-2 monolayers were created by seeding cells onto permeable, tissue culture treated membranes with a growth area of 0.33cm^2 and a 0.4µm pore size (Transwell® permeable supports, Corning Inc., NY, USA) at a 2.4 x 10^5 cells /cm^2 density[20] for 21 days, before further exposure.
Baseline transepithelial electrical resistance (TEER) was measured before each experiment, using an EVOM2 epithelial volt-ohmmeter and a STX2 chopstick electrode (World Precision Instruments, Sarasota, FL, USA). Hereafter, cells were basolaterally exposed to 10, 25, or 100 µM omeprazole, diclofenac, or a combination of both (all Sigma-Aldrich, St. Louis, MO, USA) for 2 hours. Drugs were diluted in PBS and DMSO. Omeprazole and diclofenac were selected as they are the most frequently prescribed PPI and NSAID, respectively, in the Netherlands. Drug solutions were added to the

basolateral compartment to mimic systemic exposure. In all solutions, the DMSO concentration was less than 0.25%, which did not affect TEER values (data not shown). TEER was measured every 30 minutes. Directly after adding the drug solutions, fluorescein isothiocyanate-dextran (FITC) 4kDa, 1 mg/mL (Sigma-Aldrich) was added to the apical side. Paracellular permeability was quantified by sampling 50 µL of medium from the basolateral compartment every 30 minutes. FITC fluorescence was measured spectrophotometrically. Unexposed monolayers were used as negative controls. All experiments were executed three times in triplicate.

Statistical analysis

A two-way repeated-measures analysis of variance was conducted to assess the impact of different drug concentrations on the proportional change in TEER or FITC permeation over time. Post-hoc comparisons, applying Bonferroni-correction, were conducted to detect differences between the different concentrations.

Ex vivo Ussing chamber experiments

To evaluate the role of host-susceptibly, colonic biopsies of MC patients and healthy controls were used in an Ussing chamber experiment for *ex vivo* analysis of epithelial barrier function.[21] For this proof of principle study only collagenous colitis (CC) cases were selected, as they showed the strongest association with NSAID and PPI exposure.
Six patients with CC in remission and six non-MC, age and gender matched healthy controls, underwent flexible sigmoidoscopy with standardized collection of 6 biopsies at 30 cm from the anal ring. No bowel preparation was given prior to colonoscopy, in order to not disturb mucosal homeostasis. Daily bowel movements were assessed with a 7-day defecation diary, prior to sample collection, in order to exclude active disease. Disease activity was assessed applying the Hjortswang criteria.[22] In addition, a stool sample was cultured for Salmonella, Shigella, Campylobacter, Yersinia and Clostridium species to exclude bacterial gastroenteritis. PPI or NSAID use up to two weeks prior to endoscopy was excluded. Patients with a reported drug-induced cause of their disease or excessive alcohol use were also excluded. Hereto, medical charts were reviewed upon inclusion. CC cases were diagnosed according to the internationally applied criteria for MC.[23]
Sampled colonic biopsies were transported to the laboratory in ice cold Krebs-Ringer bicarbonate (KRB) buffer within 15 minutes after retrieval. Thereafter, they were mounted directly in 1.5 mL Ussing chambers with an exposed tissue area of 1.76 mm^2 as described by Wallon *et al.*.[21] The chambers were continuously oxygenated, and a pH 7.4 and temperature of 37°C were maintained. After an equilibration phase of 40 min, the 120 min experiment started. TEER, trans-epithelial potential difference (PD), and short-circuit current (Isc) were recorded. Biopsies with a baseline TEER below 15 Ωcm^2, or between 15-20 Ωcm^2 in combination with a PD>-0.5 mV were excluded for further analyses.[21] Serosal compartments were exposed to double physiological concentrations

of omeprazole (10 µM), diclofenac (15 µM) or both.[24,25] Untreated samples served as negative control. Paracellular permeability was assessed by permeation of fluorescein sodium salt (1 mg/ml).

Statistics

Baseline values were reported as medians, with interquartile rage (IQR). Between group differences in TEER were analyzed using Mann-Whitney-U. Within group differences were assessed by Wilcoxon signed rank's test. A p-value <0.05 was considered statistically significant.

Ethical considerations

This study was executed according to the Declaration of Helsinki 2013. Conductance of the Ussing chamber experiments was approved by the medical ethical committee azM / UM, Maastricht, the Netherlands (NL48505.068.14) and was registered on ClinicalTrials.gov (NCT02303132). Written informed consent was obtained from all subjects prior to participation.

Results

Associations between MC and NSAID / PPI exposure

In total, 1,492 PALGA registered patients of 18 years or older, diagnosed with MC, CC, or LC between 2000-2012, were linked to the PHARMO Database Network. Of those, 307 had an index date before their inclusion in the PHARMO Database Network and 67 patients had less than 1 year of data collection before the index date. The remaining 1,118 cases were matched to 5,590 non-MC controls. The average age at index date was 61.0 ± 14.7 years for the cases and 61.6 ± 14.0 years for the controls (p>0.05). In both groups 74.8% was female. In the case population, at least one prescription of NSAIDs or PPIs was ever registered before index date in 70.3% and 49.5% of MC patients, respectively. In the control group the corresponding percentages were 54.5% and 30.9%.

Current (adjusted Odds ratio (AOR) 2.27, 95% CI 1.56-3.28) and recent (AOR 1.96, 95% CI 1.39-2.76) use of NSAIDs and current (AOR 2.26, 95% CI 1.70-2.99) and recent (AOR 2.99, 95% CI 2.31-3.88) use of PPIs was significantly associated with MC compared to never use. Highest risks of MC were observed for concomitant exposure to NSAIDs and PPIs (Table 5.1), especially in case of recent co-exposure (AOR 5.89; 95% CI 3.88-9.18).

In PPI users, the difference between current or recent use versus past use, was also statistically different. No statistical difference was observed between current and recent use of NSAIDs or PPIs (Table 5.1). No dose-dependent relationship was present and a

13-36 month or 4-12 month continuous exposure to NSAIDs or PPIs, respectively, was associated with the highest risk of MC (Supplemental Table S5.1).

Table 5.1 Exclusive and concomitant use of NSAIDs and/or PPIs and the risk of MC, by recency of use

	Cases		Controls		Crude	Adjusted[a]
	n=1,118	%	n=5,559	%	OR (95% CI)	OR (95% CI)
NSAID use before index date*						
Never use of NSAIDs	331	29.6	2445	45.5	1.00	1.00
Exclusive NSAID use						
Past NSAID use	393	35.2	1715	30.7	1.90 (1.61-2.24)	1.55 (1.31-1.84)
Recent NSAID use	54	4.8	178	3.2	2.44 (1.74-3.40)	1.96 (1.39-2.76)
Current NSAID use	48	4.3	138	2.5	2.94 (2.05-4.20)	2.27 (1.56-3.28)
Non-exclusive NSAID use	291	26.0	1014	18.1	2.43 (2.02-2.92)	1.85 (1.53-3.28)
PPI use before index date*						
Never use of PPIs	565	50.5	3860	69.1	1.00	1.00
Exclusive PPI use						
Past PPI use	64	5.7	274	4.9	1.66 (1.25-2.21)	1.36 (1.01-1.84)
Recent PPI use	111	9.9	221	4.0	3.71 (2.89-4.77)	2.99 (2.31-3.88)
Current PPI use	87	7.8	221	4.0	2.96 (2.26-3.89)	2.26 (1.70-2.99)
Non-exclusive PPI use	291	26.0	1014	18.1	2.14 (1.81-2.53)	1.75 (1.47-2.08)
Concomitant NSAID and PPI use						
Never use of NSAIDs or PPIs	245	21.9	651	39.9	1.00	1.00
Concomitant NSAID and PPI use						
Past concomitant use	220	19.7	893	15.9	2.52 (2.04-3.10)	2.22 (1.79-2.75)
Recent concomitant use	40	3.6	60	1.1	6.87 (4.47-10.6)	5.89 (3.88-9.18)
Current concomitant use	31	2.8	61	1.1	5.03 (3.17-7.97)	4.30 (2.68-6.92)
Non-concomitant NSAID and PPI use	582	52.0	2346	42.0	2.45 (2.08-2.89)	2.22 (1.87-2.62)

OR odds ratio, *CI* confidence interval, *NSAID* non-steroidal anti-inflammatory drug, *PPI* proton pump inhibitor. Recency of use was defined as the last prescription 61-90 (current use), 91-150 (recent use), >150 (past use) days before index date. * Exposure was defined as exclusive use of NSAIDs or PPIs, any concomitant exposure to the other drug class was excluded. [a] Adjusted for PPI and SSRI use [NSAID group], NSAID and SSRI use [PPI group] or SSRI use [NSAID+PPI group], defined as any exposure to these drugs in the 6 months prior to index date

In vitro cell culture experiments

For all diclofenac concentrations, TEER values were significantly lower in exposed versus control Caco-2 cell monolayers (all $p<0.01$). Moreover, the effect on TEER was found to be dose-dependent (Figure 5.1A). After exposure to omeprazole, TEER values were also significantly lower compared to control (all $p<0.02$), but no differences were observed between 10, 25 and 100 µM (Figure 5.2A). When cells were co-exposed to 10, 25 or 100 µM of both omeprazole and diclofenac, results comparable to omeprazole exposure were observed (Figure 5.3A). However, no significant changes were found in FITC permeation for any of the drug concentrations alone or in combination when compared to control (Figure 5.1B-5.3B).

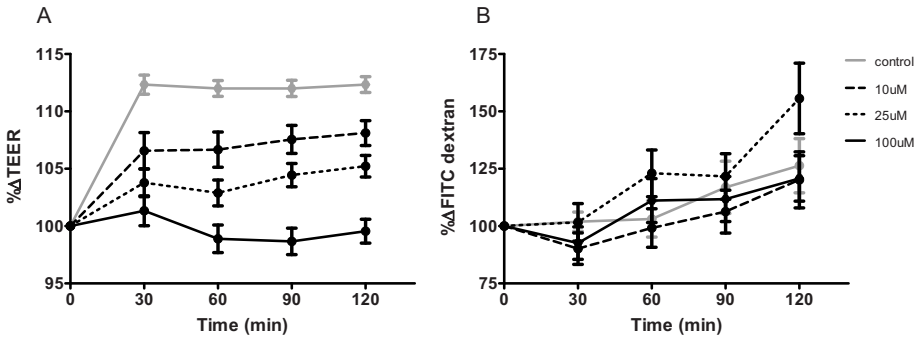

Figure 5.1 Percentage of change in transepithelial electrical resistance (TEER) (A) and permeation of fluorescein isothiocyanate-dextran (FITC) 4kDa (B) over time after exposure to diclofenac. Measurements performed in CaCo-2 cell monolayers

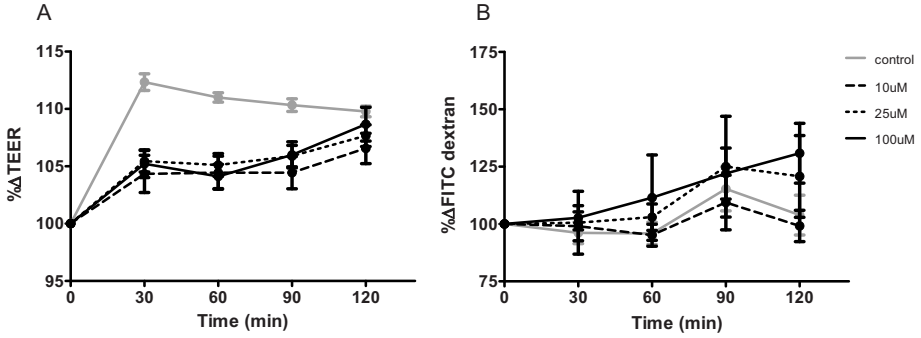

Figure 5.2 Percentage of change in transepithelial electrical resistance (TEER) (A) and permeation of fluorescein isothiocyanate-dextran (FITC) 4kDa (B) over time after exposure to omeprazole. Measurements performed in CaCo-2 cell monolayers

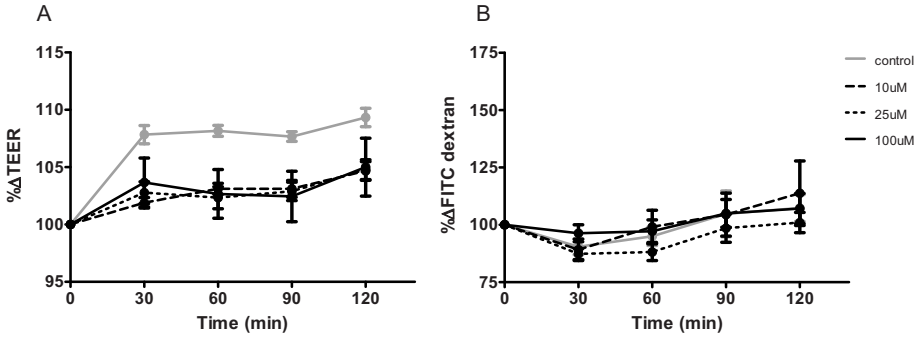

Figure 5.3 Percentage of change in transepithelial electrical resistance (TEER) (A) and permeation of fluorescein isothiocyanate-dextran (FITC) 4kDa (B) over time after co-exposure to similar concentrations of diclofenac and omeprazole. Measurements performed in CaCo-2 cell monolayers

Ex vivo Ussing chamber experiments

In both case and control group, five males and one female were included. The average age was 66.5 ± 9.4 years for the cases and 66.8 ± 10.8 years for the controls (p>0.05). For cases, the median disease duration was 14 months (range 9-37 months) and the median duration of clinical remission was 10 months (range 5-13 months). None of the subjects fulfilled the criteria for active disease or had gastroenteritis based on a positive stool culture at time of inclusion. Four out of six cases used budesonide 3 mg/day as maintenance therapy.

In total, 72 biopsies were collected, of which seven (10%; *i.e.* 4 in the cases and 3 in the controls) were excluded because baseline TEER/PD values did not match the inclusion criteria. The median TEER at baseline was 31 (IQR 24-46) Ωcm^2 for the CC cases and 31 (IQR 28-45) Ωcm^2 for the non-MC controls (p>0.05). In the cases, the change in TEER and the permeation of fluorescein were not statistically different after exposure to any of the drug solutions, when compared to non-MC controls; nor at any time point, when compared to unexposed biopsies (Figure 5.4, 5.5). No differences regarding the change in Isc over time were observed between groups or type of drug exposure (data not shown).

Discussion

In this triptych on NSAID and PPI induced MC, we aimed to confirm the association with concomitant exposure to these drugs, to study their direct effect on the colonic paracellular permeability, and to explore the role of host-factor susceptibility in drug-induced MC. The results of the observational part of this study confirmed the association between NSAIDs, PPIs, and above all, co-exposure and MC. However, a direct effect of those drugs on the paracellular permeability of CaCo-2 cells or the biopsies of MC patients versus non-MC controls was not observed.

The associations between PPIs, and especially NSAID-PPI co-exposure, as observed in the Dutch population, were similar to those reported in a previous study of our group, performed in the British CPRD.[1] The current associations for NSAIDs were stronger than those found in the CPRD population. Theoretically, variation in the strength of the associations might be explained by varying methodology such as differences in uncorrected confounders (*e.g.* smoking, undocumented drug use), case ascertainment, or prescription policy between the two countries (*e.g.* preference to prescribe a specific drug of a drug class, which might be more associated to MC[15]). Moreover, correction for concomitant exposure (being PPIs in NSAID users, and vice versa) can be performed by applying statistics, or, by full exclusion of any concomitant use based on prescription data, as performed in the current study. This will better reflect a 'true association' with MC. Despite methodological differences, the observed associations were in line with recent findings.[2,3]

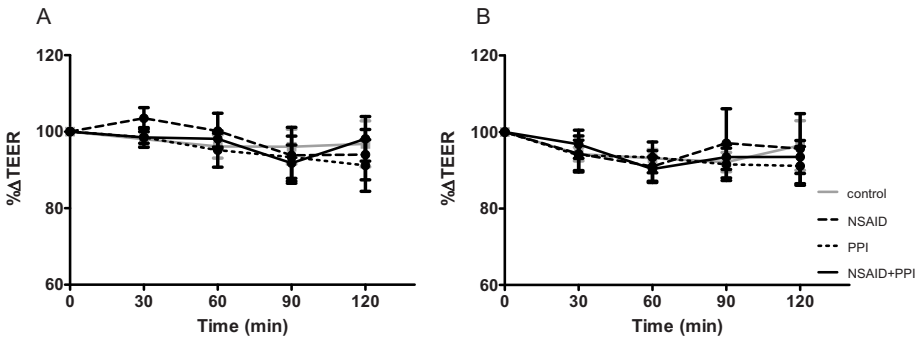

Figure 5.4 Percentage of change in transepithelial electrical resistance (TEER) over time after exposure to diclofenac (NSAID) 15µM, omeprazole (PPI) 10 µM, or a combination of both, in fresh colonic tissue of MC cases (A) and non-MC controls (B) mounted in Ussing chambers

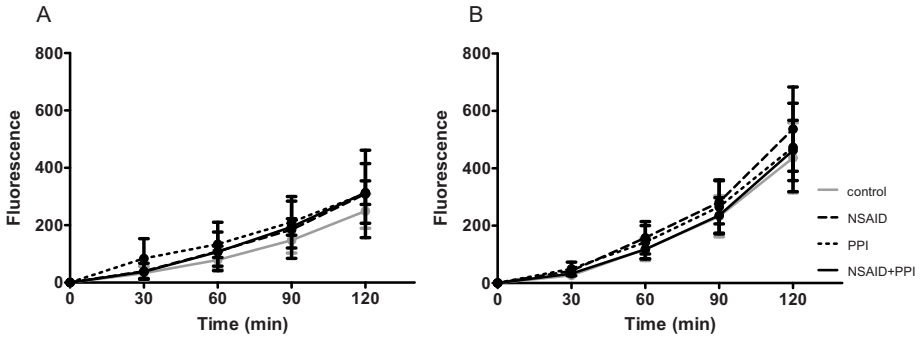

Figure 5.5 Fluorescence of fluorescein over time, after exposure to diclofenac (NSAID) 15µM, omeprazole (PPI) 10 µM, or a combination of both, in fresh colonic tissue of MC cases (A) and non-MC controls (B) mounted in Ussing chambers. Fluorescein fluorescence was measured spectrophotometrically

So far, the concept of drug-induced MC is mainly based on observational data, supported by a limited number of clinical case-reports. All these studies described a pronounced association of MC with single and concomitant exposure to NSAIDs or PPIs, justifying further research on possible underlying mechanisms. To our knowledge, only hypotheses exist on possible pathophysiological mechanisms of drug-induced MC, of which a direct compromising effect on the colonic epithelial barrier by affecting tight junction and/or cytoskeletal structures, has most frequently been mentioned.[11,26]
NSAIDs and PPIs are generally administered orally and are well absorbed in the upper gastrointestinal tract. Moreover, NSAIDs and PPIs mainly undergo renal clearance. However, some NSAIDs (*e.g.* diclofenac, indomethacin) undergo enterohepatic recirculation and are reabsorbed in the ileum after being partially excreted in bile as

unchanged drug or inactive metabolites.[27,28] Therefore, luminal concentrations in the colon are expected to be lower than basolateral concentrations. Therefore, basolateral exposure based on supra-physiological concentrations was investigated for these proof-of-principle experiments. Furthermore, experiments were performed with the most frequently prescribed NSAID and PPI in the Netherlands.

To study a potential direct effect of NSAIDs and PPIs on paracellular permeability, we used CaCo-2 cell monolayers. In diclofenac treated cells, all concentrations (10-100 µM) did result in lower TEER values compared to the untreated condition, at all time points (Figure 5.1A). However, FITC permeation of NSAID exposed cells did not differ from control, indicating that paracellular permeability was not affected. This finding is contradictive to the results of studies reporting on increased paracellular permeability in indomethacin exposed CaCo-2 cells.[29,30] These studies however, exposed the more sensitive apical side to higher dosages (250-1000µM). Furthermore, indomethacin might have a more pronounced inhibitory effect on the paracellular permeability, compared to diclofenac.[31] The observed increase in TEER in the absence of increased marker permeation, may point to altered ion fluxes. Theoretically, this might be due to the ability of NSAIDs to uncouple oxidative phosphorylation, impeding the maintenance of the electrolyte balance due to reduced cellular energy levels.[26,32]

In omeprazole treated cells paracellular permeability was maintained as well, as no effect was observed on FITC permeation. The lower TEER values observed in omeprazole treated cells might also be due to changes in the flux of electrolytes (Figure 5.2A). It is known that CaCo-2 cells possess an H^+/K^+-ATPase.[33] In theory, these drugs might therefore also influence the gastric-like proton pumps *in vivo,* which are suggested to be present in the colon.[34,35] However, the luminal pH of the colon is considered too alkaline to induce notable activation of any unchanged drug reaching the colon.[36] However, confirmative data on fecal concentrations of metabolized or unchanged PPIs are unavailable. In contrast to our hypothesis, co-exposure to supra-physiological concentrations of diclofenac and omeprazole did not compromise the paracellular permeability in Caco-2 monolayers.

To check these findings in a more physiological mode and to further evaluate involvement of host-factor susceptibility, fresh colonic biopsies of CC patients and non-MC controls were exposed basolaterally to MC associated drugs *ex vivo*. To be able to detect a potential effect, human peak plasma concentrations of diclofenac and omeprazole were doubled.[24,25] Only CC patients in remission were included as patients with active disease may already exhibit a disrupted epithelial barrier function due to the inflammatory process[37,38] and drug associations were stronger for CC than LC. In line with the *in vitro* experiments, Ussing chamber experiments showed no effect of diclofenac, omeprazole, or both on the paracellular permeability of healthy controls or CC cases. These findings suggest that a direct effect of NSAIDs and PPIs on colonic paracellular permeability is unlikely to be the key mechanism of drug-induced MC.

However, an effect on TEER was absent as well, which was in contrast to the *in vitro* studies. The epithelium of fresh colonic tissue is more 'leaky' in comparison to CaCo-2 models, which might have impeded detection of small changes in transcellular electrophysiology. Despite, these findings, indirect effects such as a compromising effect on barrier function by changes in the intestinal microbial composition, small bowel physiology, or bile acid metabolism cannot be excluded. With regard to concomitant NSAID and PPI exposure, a 'two-hit hypothesis' with PPI related microbial perturbations exacerbating NSAID-induced mucosal changes has been suggested.[39] This would be an explanation for the high risk of MC in case of concomitant use, observed in this and a previous study.[1] Furthermore, the exposure characteristics of PPI users with the highest risk of MC (*i.e.* current or recent users with a continuous duration of use of 4-12 months) would fit this hypothesis.[1]

Although the number of included cases was limited, random MC patients seem not to be a susceptible host for drug-induced MC, per definition. This supports epidemiological data describing a relatively small proportion of MC patients with a drug-induced disease.[40] As in post-infectious irritable bowel syndrome, research on *e.g.* gene polymorphisms and gene expression alterations, related to epithelial integrity associated pathways, might be of interest to explore host-susceptibility in MC in future.[41,42]

Four out of six cases used oral budesonide 3 mg/day maintenance therapy since 13-15 months. Münch *et al.* observed a residual barrier disruption might remain 6 weeks after achievement of clinical remission with oral budesonide.[37] However, this might be due to residual pro-inflammatory cytokines. It is unknown whether the barrier disruption lasts after a long period of clinical remission. Nevertheless, baseline TEER values of patients and controls were comparable to those in the study of Münch *et al.*[37] and did not differ between the two groups. Furthermore, 5 out of 6 CC cases included in the Ussing experiments were male, whereas MC is a predominantly female disorder. However, there are no indications to assume a difference in electrophysiology and functioning of the tight junction complex between males and females.

Retrospectively, exposure to NSAIDs and PPIs is strongly associated with MC. However, a direct compromising effect of these drugs on the paracellular permeability, or a general host-susceptibility among MC patients, seems to be unlikely based on our results. However, any (in)direct effects of these drugs on other aspects of colonic barrier function (e.g. transcellular permeability, mucosal barrier) cannot be excluded. To better understand whether barrier function plays a crucial role in drug-induced MC, or why and how a small population develops it, registration of well-described and prospectively followed cases of drug-induced cause MC, preferably with collection of biomaterials, is warranted. Withdrawal, preferably followed by rechallenge of the suspected drug, without interference of simultaneously started anti-inflammatory treatment is then of relevance.

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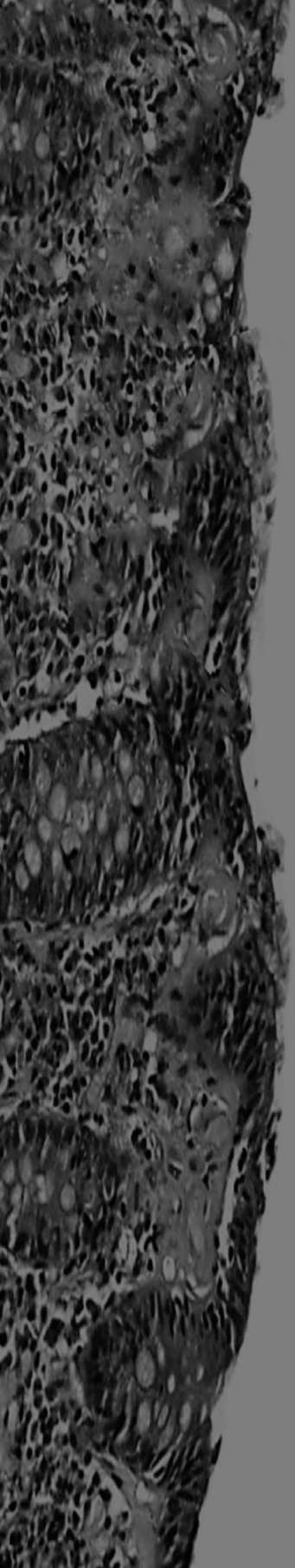
Supplemental table

Table S5.1 Current use of NSAIDs or PPIs and the risk of MC, by average daily dose and duration of use

	Cases		Controls		Crude	Adjusted[a]
	n=1,118	%	N=5,559	%	OR (95% CI)	OR (95% CI)
NSAID use before index date*						
Current use	48	4.3	138	2.5		
By average daily dose						
One prescription only	2	4.2	13	9.4	1.17 (0.34-4.00)	1.05 (0.29-3.84)
Low (<0.75 DDDs)	25	52.1	88	63.8	1.82 (1.24-2.67)	1.26 (0.84-1.88)
Medium (0.75-1.25 DDDs)	13	27.1	17	12.3	5.63 (3.08-10.31)	3.50 (1.84-6.66)
High (>1.25 DDDs)	8	16.7	20	14.5	3.07 (1.75-5.39)	1.63 (0.90-2.96)
By duration of use						
<3 months	31	64.6	115	83.3	1.78 (1.26-2.52)	1.26 (0.88-1.82)
3-12 months	9	18.8	14	10.1	4.03 (2.17-7.47)	2.32 (1.21-4.47)
13-36 months	6	12.5	5	3.6	8.45 (3.54-20.18)	5.77 (2.31-14.35)
>36 months	2	4.2	4	2.9	6.01 (2.10-17.20)	2.28 (0.75-6.90)
PPI use before index date*						
Current use	87	7.8	221	4.0		
By average daily dose						
One prescription only	2	2.3	6	2.7	1.91 (0.62-5.88)	1.45 (0.46-4.59)
Low (<0.75 DDDs)	35	40.2	92	41.6	2.44 (1.73-3.45)	1.69 (1.17-2.42)
Medium (0.75-1.25 DDDs)	26	29.9	66	29.9	2.52 (1.64-3.86)	2.01 (1.29-3.14)
High (>1.25 DDDs)	24	27.6	57	25.8	2.88 (1.89-4.37)	1.95 (1.25-3.02)
By duration of use						
<3 months	28	32.2	83	37.6	1.74 (1.19-2.54)	1.19 (0.80-1.78)
3-12 months	27	31.0	45	20.4	3.94 (2.58-6.04)	2.83 (1.82-4.42)
13-36 months	14	16.1	37	16.7	2.93 (1.71-5.01)	2.30 (1.32-4.01)
>36 months	18	20.7	56	25.3	2.65 (1.65-4.25)	1.92 (1.17-3.15)

OR odds ratio, *CI* confidence interval, *NSAID* non-steroidal anti-inflammatory drug, *PPI* proton pump inhibitor, *DDD* defined daily dosages. Current use was defined as the last prescription 61-90 days before index date. * Exposure was defined as exclusive use of NSAIDs or PPIs, any concomitant exposure to the other drug class was excluded. [a] Adjusted for PPI and SSRI use [NSAID group] or NSAID and SSRI use [PPI group], defined as any exposure to these drugs in the 6 months prior to index date



6

Early life exposure, lifestyle and co-morbidity as risk factors for microscopic colitis: a case-control study

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Abstract

Background

The pathophysiology of microscopic colitis (MC) is not fully understood. A dysregulation of the adaptive immune response has been hypothesized, of which the maturation and function is imprinted in early life. Various other factors (e.g. hormonal factors) have also been found to be associated, sometimes with minimal or conflicting evidence. The aims of this study were to evaluate whether an exposure to (microbial) agents in early life might be protective for MC development, and to assess the role of several less well-established risk factors in one study.

Methods

A case-control study was performed including MC cases diagnosed in the southern part of the Netherlands between 2000-2012. Cases were matched to non-MC controls from the same area, based on gender and year of birth, and assigned the same index date. All subjects filled out the same study questionnaire on various risk factors.

Results

In total, 171 MC cases and 361 controls were included. In the multivariable logistic regression analysis, current smoking (OR 6.23, 95%CI 3.10-12.49), arthrosis and a cardiac disorder were associated with MC. No association was observed for e.g. factors related to early life exposure to microbial antigens, passive smoking, rheumatoid arthritis, celiac disease or hormonal factors.

Conclusion

Early life exposure to microbial antigens and hormonal exposure were not associated with MC. Current smoking seems to be an incontestable risk factor for MC. Therefore, exposure to environmental risk factors in later may be of relevance in MC pathogenesis and warrants further investigation.

Introduction

Over the last decades, the incidence of microscopic colitis (MC) has increased globally. Worldwide, pooled incidence rates of 4.14 and 4.85 per 100,000 person years have been reported for collagenous colitis (CC) and lymphocytic colitis (LC), respectively.[1] The condition is predominantly found in elderly (60+ years) and women. Clinically, patients with MC suffer from chronic or intermittent watery diarrhoea, which significantly influences patients' health-related quality of life.[2]

The underlying pathophysiological mechanism of the disease is still not fully understood, but is likely to be multifactorial. Penetration of luminal agents through a disrupted epithelial barrier, eliciting a local immune response in genetically predisposed host is often considered an important factor in MC pathogenesis.[3,4] Indications for a disrupted barrier function were reported by Münch *et al.*, who observed a significant drop in transepithelial resistance, in conjunction with an increased translocation of non-pathogenic bacteria, in both active and remission MC patients compared with non-MC controls.[4,5] It is however not clear whether this is a primary or secondary effect and what would be the exact trigger for a disrupted colonic barrier. Examples of luminal agents that have been associated with MC are bile acids and drugs.[6-8] Especially (concomitant) exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) is associated with an increased risk of MC.[9,10] However, not every patient with MC has a drug-induced cause of the disease and only a limited number of NSAID/PPI users develop MC. In addition, regular alcohol consumption and smoking have been found to be associated with MC.[11-14] Although the link between MC and (especially current) nicotine exposure is currently well-documented, no studies addressed the effect of passive nicotine exposure or potential exposure to other hazardous substances on the risk of MC.

Other risk factors linked to MC are a cholecystectomy or appendectomy prior to diagnosis, or familial occurrence of MC. However, the studied populations were of limited size.[15-17] One other study assessed the effect of female sex hormones on the risk of MC, which is of interest considering the female predominance and high prevalence in elderly. However, the study showed conflicting findings and contained methodological shortcomings.[18] Another study addressed the effect of socio-economic aspects on the MC risk, but found contradictive results as MC was more prevalent in areas with more tertiary educated (*i.e.* college or university) inhabitants as well as in areas with lower incomes and housing values.[19]

Most studies tend to focus on a selection of risk factors. Those investigating the association between various comorbidities and MC, repeatedly showed a higher prevalence of autoimmune comorbidities (*e.g.* rheumatoid arthritis, thyroiditis, celiac disease).[20-22] Although the exact mechanism of this association is unclear, it indicates involvement of the immune system in MC pathogenesis. Genetically, the association with, for example, celiac disease is supported by an increased prevalence of celiac

disease related HLA-DQ haplotypes (*i.e.* HLA-DQ2 or HLA-DQ1/3) in MC compared with non-MC subjects.[23-25] Although there is no concrete immunological evidence to support an autoimmune aetiology, other immunological differences have been observed. For instance, increased numbers of $CD8^+$ T-cells and regulatory T-cells were found in the mucosa of patients with MC.[26] Furthermore, cytokine profiles in MC patients showed a mixed Th1/Tc1 and Th17/Tc17 profile, which may imply a dysregulation of the adaptive immune response.[27] The maturation and function of this response is imprinted by exposure to microorganisms in early life. According to the so called 'hygiene hypothesis', a more protective and sanitary upbringing in childhood might affect immune maturation and development and consequently increase the risk of an inappropriate immunologic responses in later life. Because of the possible parallel with inflammatory bowel disease (IBD), where an association with the hygiene hypothesis has been reported,[28] it would be of interest to assess whether factors associated with a decreased early life microbial exposure increase the risk of MC.

The primary aim of this study was to assess potential risk factors for MC, with special focus on factors involving early life microbial exposure, passive nicotine exposure and female hormone exposure, to generate possible new insights in MC pathophysiology. Second, this study aimed to combine them with other established and potential MC risk factors in one study, to assess their association with MC.

Methods

Case definition

A case-control study was conducted, including MC cases from all 10 hospitals (9 local hospitals and 1 referral center) in the southeastern part of the Netherlands. MC cases were identified using PALGA, the Dutch nationwide registry of histopathology- and cytopathology, which reached full national coverage from 1991 onward.[29] First, cases were selected if 1) they had a PALGA registered diagnosis of MC, CC or LC in one of the participating clinical centres between January 2000 and December 2012, 2) they were still alive and 3) were aged 18 years or older at time of diagnosis. Second, cases in which a diagnosis of MC could not be confirmed based on the available pathology reports and medical charts, were excluded in advance. The remainder was invited to participate in the study. On inclusion, pathology slides of participating cases were carefully revised according to the accepted diagnostic criteria for MC[30] by an experienced pathologist (D.G.), if available. Cases not fulfilling these criteria were still excluded from participation. The index date for the cases was defined as the date of histological diagnosis.

Control definition

Non-MC controls were retrieved from a large research cohort of more than 1,650 randomly selected inhabitants (>18 years old) of South Limburg. All non-MC subjects which had given consent to be approached for future studies, were informed about the study. Participants were matched to the included cases on a 2:1 ratio, based on gender and year of birth (±2 years). Non-MC controls were assigned the same index date as their matched case.

Questionnaire

All subjects were asked to complete a questionnaire specially designed for this study. The questionnaire contained questions regarding: age, gender, educational level (classified as 0-7 according to the Dutch educational system, ranging from no education (0) to a university degree (7)), self-reported comorbidities with diagnosis year (only pre-MC comorbidities were included), alcohol use, exposure to hazardous substances at work, smoking status, passive nicotine exposure (parental or partner smoking and nicotine exposure at work) and markers related to early life exposure to microbial antigens (number of siblings, birth order, day care attendance, pets, breastfeeding, vaccination, and frequent use of antibiotics in childhood). Women were asked about hormonal factors (age at menarche and/or menopause, use of oral contraceptives or hormones, and pregnancies).

Ethical approval

This study was conducted in line with the revised version of the Declaration of Helsinki and was approved by the medical ethical committee of Maastricht University Medical Center^{+} (NL44127.068.13). The study was also registered at ClinicalTrials.gov (NCT01928667). Written informed consent was obtained from all participants.

Statistical analyses

Descriptive statistics were used to express frequencies and proportions. For continuous variables, mean with standard deviation (SD) or median with interquartile range (IQR), were calculated, depending on the normality of the distribution. Differences between groups were calculated using Student's T-test or Mann-Whitney-U test. Univariate logistic regression analysis, adjusted for age and gender, was applied to calculate odds ratios (OR) with 95% confidence intervals (95% CI). Variables with a p-value <0.10 in the univariate analyses were included in the multivariable logistic regression analysis. The presence of multicollinearity between selected variables was assessed by requesting collinearity diagnostics in the statistical program. To be able to detect a minimal difference in proportions of 0.15, which was considered clinically relevant, at least 75 cases per MC subtype were required. Statistical significance was determined as $p<0.05$.

Analyses were performed with IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Study population

As outlined in Figure 1, a total of 821 cases with a diagnosis of MC, within the designated time window and catchment area, were retrieved from PALGA. Of those, 555 patients (67.6%) did meet the first and second selection criteria and were invited to participate in the study. Hereof, 192 subjects agreed to participate (34.6%). After receiving the questionnaire, 4 subjects (2.1%) decided not to participate, 10 subjects (5.2%) never returned the questionnaire, and the content of 1 questionnaire was considered unreliable because of unclear, discrepant, and unreadable answers (0.5%). Six more cases (3.1%) were excluded as they did not fulfil the criteria for any subtype of MC based on the biopsy revision. In total, 171 MC cases (79% women; mean age 55.5 ± 11.3 years) were included for further analyses. On biopsy revision, cases were categorized as CC (n=69, 40.4%), LC (n=65, 38.0%), or incomplete MC (MCi) (n=17, 9.9%). In 32 (21%) cases, the subtype was different than reported in the pathology report, which was mainly due to the fact thatcases could also be classified into the new subtype (MCi)[30] on revision. Biopsy slides of the remaining 20 cases (11.7%) could not be reviewed because they could not be retrieved or were of insufficient quality. In those cases, the PALGA registered diagnosis was retained. Based on the pathologist's report and conclusion, a reasonable to high likelihood of fulfilling the diagnosis of MC was present in all these cases.

A group of 444 non-MC controls agreed to participate in this study and returned the questionnaire. Considering the age and gender distribution of the cases in respect to the available controls, 316 non-MC controls could be matched to the MC cases based on gender and year of birth.

Subject characteristics

Age and gender did not differ between both groups (Table 6.1). Based on the univariate analyses on other subject characteristics and self-reported comorbidities, a lower educational level as well as cardiac disease, non-asthmatic pulmonary disorder, gastric disorder, liver disorder, depressive disorder, arthrosis, chronic back pain, rheumatoid arthritis, esophageal disorder and celiac disease (all before the index date) were found to be significantly associated with an increased risk of MC (Table 6.2 and Supplementary Table S6.1). No increased risk was found for having a thyroid disorder or diabetes mellitus before index date, familial occurrence of MC, or having a partner with MC.

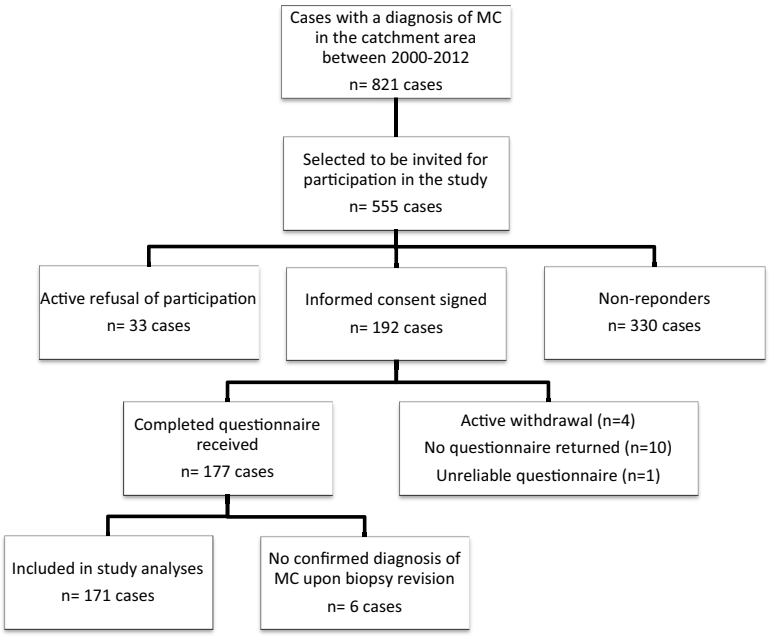

Figure 6.1 Flow-chart of the selection process and participation rates of the case population. MC: Microscopic colitis

Table 6.1 Subject's characteristics

	MC cases (n=171)	Non-MC controls (n=316)
Females (%)	138 (80.7%)	250 (79.1%)
Age at index date in years (mean ± SD)		
Overall	57.1 ± 11.7	56.1 ± 11.3
Men	59.7 ± 11.7	60.0 ± 12.0
Women	56.5 ± 11.6	55.0 ± 10.9
Caucasian ethnicity (%)	165 (98.8%)	298 (98.7%)
MC subtype (%)		
Collagenous colitis	81 (47.4%)	
Lymphocytic colitis	73 (42.6%)	
Incomplete MC	17 (10.0%)	

MC: Microscopic Colitis, SD: standard deviation

Factors related to early life microbial exposure

Patients with MC did not more frequently grow up without siblings, nor did the total number of siblings or the birth order within their family differ from that of non-MC controls. Furthermore, the number of cases and controls that had attended day care, had any allergies, received breastfeeding, was vaccinated or did use antibiotics in

childhood was not statistically different between groups (see Supplementary Table S6.1). In contrast, patients with MC did more often have pets (especially cats and/or dogs) at home in their childhood (Table 6.2 and Supplementary Table S6.1).

Table 6.2 Possible risk factors for MC. Statistically significant results (p≤0.10) of the univariate analyses

	MC Cases (n=171)	Non-MC Controls (n=316)	Adjusted Odds Ratio (95% CI)*
Smoking status[#]			
Current smoker	65 (38.7%)	40 (12.9%)	5.54 (3.24-9.47)
Smoking status partner[#]			
Current smoker	35 (20.5%)	46 (14.6%)	1.66 (0.97-2.85)
Nicotine exposure at work[#]			
Current exposure	14 (8.2%)	9 (2.8%)	2.75 (1.14-6.63)
Educational level (continuous)			0.88 (0.79-0.99)
Cardiac disease[#]	23 (13.5%)	22 (7.0%)	1.94 (1.03-3.67)
Non-asthmatic pulmonary disorder[#]	25 (14.6%)	26 (8.2%)	1.69 (0.94-3.05)
Gastric disorder[#]	20 (11.7%)	18 (5.7%)	1.95 (1.00-3.82)
Liver disorder[#]	11 (6.4%)	5 (1.6%)	3.97 (1.35-11.70)
Depressive mood disorder[#]	38 (22.2%)	34 (10.8%)	2.16 (1.29-3.61)
Arthrosis[#]	62 (36.3%)	53 (16.8%)	2.53 (1.60-4.00)
Chronic back pain[#]	81 (47.4%)	114 (36.1%)	1.40 (0.95-2.08)
Rheumatoid arthritis[#]	22 (12.9%)	17 (5.4%)	2.27 (1.16-4.46)
Esophageal disorder[#]	8 (4.7%)	2 (0.6%)	7.22 (1.50-34.85)
Celiac disease[#]	6 (3.5%)	0 (0.0%)	10.86 (1.29-91.33)[§]
Presence of animals at home[&]	135 (78.9%)	223 (70.6%)	1.61 (1.03-2.50)
Female subjects only	MC Cases (n=138)	Non-MC Controls (n=250)	
Number of pregnancies (mean ± SD) [#]	2.3 ± 1.4	1.90 ± 1.2	1.23 (1.04-1.44)
Use of hormonal supplements[#]	30 (21.7%)	31 (12.6%)	1.89 (1.08-3.29)

MC: Microscopic colitis; SD: standard deviation; 95% CI: 95% Confidence Interval. [#] before the index date
[&] in childhood. [^] excessive alcohol use was defined as ever continuous use of >21 units per week for more than months. * Adjusted for age and gender. [§] Calculated with at least one positive subject in either the case or control group

Nicotine exposure, alcohol use and hazardous substances

As shown in Table 6.2 and Supplementary Table S6.1, current smokers were found to have a 5.54 (95% CI 3.24-9.47) times increased risk of MC compared with never smokers. After cessation of smoking, this risk dropped almost 4-fold (former versus current smokers; OR 0.26 95%CI 0.16-0.43). The duration of nicotine exposure enhanced the risk of MC (OR 1.03; 95% CI 1.02-1.06 per year). Furthermore, the average age at diagnosis was 52.8 ± 9.7 years versus 59.8 ± 12.1 years (p<0.001) for smoking vs. non-smoking at index date, respectively. Passive smoking at work at the index date was also associated with MC (OR 3.05; 95% CI 1.29-7.22). No association was observed for subjects who had 1 or 2 smoking parents, were exposed to hazardous substances at work, or reported a period of excessive alcohol use (defined as >21 units / week for a period longer than 3 months) before the index date.

Hormonal factors

In the female subjects (138 cases and 250 controls), hormonal factors were evaluated. As shown in Supplementary Table S6.1, the percentage of subjects that used oral contraceptives before the index date, had ever been pregnant before the index date, or was post-menopausal, was not different between cases and controls. The average age of menarche and menopause did not statistically differ either. By contrast, the average number of pregnancies before the index date was significantly higher in the cases vs. controls, *i.e.* 2.3 ± 1.4 versus 1.9 ± 1.3 (p=0.013) respectively (Table 6.2). Moreover, the use of hormonal supplements (*e.g.* estrogens) before the index date did significantly differ between cases and controls, *i.e.* 17.5% versus 9.8% (p=0.025), respectively.

Multivariable analysis

All univariately associated factors (i.e. with p≤0.10) were included in the multivariable model. Based on this model, 4 factors were associated with an increased risk of MC, being current smoking (OR 6.23, 95%CI 3.10-12.49), self-reported cardiac disorder (OR 3.31, 95%CI 1.31-8.38), non-asthmatic pulmonary disorder (OR 2.29, 95%CI 1.05-5.02) and arthrosis (OR 2.23, 95%CI 1.15-4.34) (Table 6.3). A non-asthmatic pulmonary disorder was no longer significantly associated with MC when only cases with revised biopsies (88.3%) were included in the analysis. In female subjects, no hormonal factors were associated with MC when included in the multivariable analysis.

Post-hoc, separate analyses were performed for LC and CC. Only cases with revised biopsy slides were included. Based on the multivariable analyses current smoking, a lower educational level and self-reported arthrosis were significantly associated with CC. In LC cases, current smoking, self-reported cardiac disease and depressive mood disorder were significantly associated with an increased risk (Table 6.3).

No multicollinearity was present among the variables included in any of the multivariable analyses.

Table 6.3 Results of the multivariable analysis

	MC Adj. OR (95% CI)*	MC – biopsy proven ^ Adj. OR (95% CI)*	CC Adj. OR (95% CI)*	LC Adj. OR (95% CI)*
Current smoking status#	6.23 (3.10-12.49)	7.30 (3.45-15.41)	14.34 (5.28-38.90)	4.27 (1.56-11.71)
Cardiac disease#	3.31 (1.31-8.38)	3.18 (1.16-8.71)	-	5.62 (1.37-23.06)
Non-asthmatic pulmonary disorder#	2.29 (1.05-5.02)	-	-	-
Arthrosis#	2.23 (1.15-4.34)	2.02 (1.00-4.11)	2.59 (1.06-6.30)	-
Depressive mood disorder#	-	-	-	2.78 (1.03-7.52)
Lower educational level	-	-	4.26 (1.48-12.28)	-

MC: Microscopic colitis; CC: Collagenous colitis; LC: Lymphocytic colitis; OR: Odds Ratio; SD: standard deviation; 95% CI: 95% Confidence Interval. # before the index date. ^ Only cases with revised biopsies included in the analyses. * Adjusted for age and gender

Discussion

The present, large case-control study evaluated various known and unknown risk factors for MC in one study. Based on the multivariable analysis in biopsy proven cases, smoking, self-reported cardiac disease and arthrosis were significantly associated with an increased risk of MC. Association with passive nicotine exposure or any factors related to early life microbial exposure was not observed in the total population. Moreover, hormonal exposure was not associated with MC in the female MC population either.

The absence of any association between MC and factors related to early life microbial exposure is in contrast to classical IBD. Our findings clearly discard the hypothesis that a more protected, sanitary upbringing might be associated with an increased risk of MC in later life. The positive association between cuddly pets (cats, dogs) in the univariate analysis is even in contrast to this hypothesis. It is known that increased exposure to microbial antigens may affect the development of the intestinal microbiota.[31] and immune system. However, based on the current findings, we cannot exclude that the intestinal microbiota may be involved in MC, as only 1 study has ever reported on microbial changes in MC[32] and extensive data on the microbial composition and activity in MC are lacking.

The observed association with current smoking in the univariable and multivariable analyses was in line with previous observations,[11-13,20] as is the finding that current smokers did develop their disease significantly more early compared to non-smoking patients.[11,12] However, in contrast to Yen *et al.,*[13] who studied a histologically less well-defined population, we found no increased MC risk in former smokers. The fact that passive nicotine exposure at home, at work or in childhood was not independently associated with MC in the multivariable analysis, indicates that low dosages of inhaled nicotine seem not to influence the risk of MC. Pathophysiologically, the strong association with active smoking might be explained by alterations in the immune response or intestinal microbiota or the negative impact on the colonic blood flow.[13,33] In contrast to current smoking, no association was observed between exposure to hazardous substances at work and MC. The effect of other aerial pollutants was not studied, but would be of interest to explore the effect of exposure polluting factors in the environment to a greater extend. With regard to alcohol consumption, no association was observed between MC and a period of excessive alcohol use before the index date. Unfortunately, data on alcohol consumption at diagnosis in general, were unavailable. Another demographic factor studied was educational level. A higher educational level was associated with a decreased risk of MC in the univariate analyses, which is just opposite to the finding of Sonnenberg *et al.*.[19] However, in the multivariable analysis, educational level was not found to be associated with MC.

Strikingly, MC was not found to be associated with a thyroid disorder, rheumatoid arthritis, or celiac disease in the multivariable analysis. This negative finding is in contrast

to previously reported associations between autoimmune disorders and MC.[20] A relevant limitation is the fact that all documented concomitant diseases were self-reported, which might have induced misclassification of comorbidities. Furthermore, the number of prevalent cases was rather low and thyroid disorders could not be specified into autoimmune and non-autoimmune variants, possibly influencing associations. The positive association between several other comorbidities (*e.g.* gastric and esophageal disorders, depressive mood disorder, chronic back pain and arthrosis) in the univariate and multivariable analyses could be due to residual confounding by MC associated drugs such as NSAIDs, PPIs, and selective serotonin reuptake
Inhibitors (SSRI).[9,34] In theory this might also account for the association with cardiac disease, in which acetylsalicylic acid frequently is prescribed.[35] Unfortunately, analyses could not be corrected for drug exposure at index date (residual confounding), for drug exposure at the index date could not be assessed reliably based on patient medical files or the study questionnaire, particularly not in the control group. This is a major drawback of this study. Uncorrected drug exposure might also partially explain the observed differences in associated comorbidities between CC and LC, as NSAID are stronger associated with CC, whereas SSRIs are more associated with LC.[20,36]
Considering the predominance of (elder) females in MC, the association between MC and hormonal factors was studied. Both estrogens and progesterone are able to reduce chemically induced colitis in animal models by reducing inflammation and improving the epithelial barrier.[37,38] Presumably, the postmenopausal decrease in sex hormone exposure might therefore elicit MC. One study addressed this topic before, but found factors related to an increased (*e.g*. young age at menarche and use of oral contraceptives) as well as a decreased hormonal exposure (*e.g.* younger menopausal age and less use of hormonal replacement therapy) to be associated with MC.[18] It should however be noted that the control group included in that study was established approximately 20 years earlier and it was not clear whether the year of diagnosis was taken into account when assessing the presence of the studied variables. In our study, hormonal factors were not associated with MC in the multivariable analysis, although the number of female cases might not have been large enough to detect small differences between populations.
Together with current and previous findings that drug exposure, changes in the gut microbiota, and bile acid malabsorption are associated with MC,[9,13,32,39] a local immunological reaction to luminal (bacterial) contents in genetically predisposed hosts, is still a plausible pathophysiological mechanism. Moreover, yet uninvestigated substances (*e.g.* viruses and air pollution) might play a role as well. Therefore, further research on risk factors for MC is warranted, as currently associated factors did not yet lead to the new leads for the possible underlying pathophysiological mechanism of MC.
A strength of this study is the combination of various known and potential risk factors for MC in 1 multivariable analysis, plus the large control group, consisting of randomly selected non-MC inhabitants of the same catchment area. Recall of historic facts might

have led to more variation in the reported answers, potentially attenuating actual differences between the 2 groups. However, recall bias was considered non-differential between groups because of matching cases and controls on year of birth.

To conclude, this study does not support the hypothesis that early life exposure to microbial agents is protective for MC development in later life. This is in contrast to the hygiene hypothesis, which is considered of relevance in IBD. Furthermore, a decreased exposure to sex hormones was not related with an increased MC risk. Current smoking was confirmed to be an incontestable risk factor for MC. Therefore, exposure to environmental risk factors in later life seems to be of relevance to MC pathogenesis and therefore needs further investigation.

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Supplemental table

Table S6.1 Univariate analyses of possible MC related risk factors

	MC Cases (n=171)	Non-MC Controls (n=316)	Adjusted Odds Ratio (95% CI)*	p-value
Smoking status[#]				
Never smoker	40 (23.8%)	131 (42.1%)	1.00	
Former smoker	63 (37.5%)	140 (45.0%)	1.42 (0.88-2.29)	0.146
Current smoker	65 (38.7%)	40 (12.9%)	5.54 (3.24-9.47)	<0.001
Missing	3 (1.8%)	5 (1.6%)		
Duration of smoking in years[#] (mean ± SD)	29.1 ± 13.8	23.5 ± 13.8	1.03 (1.02-1.06)	<0.001
Duration of stopped smoking in years[#] (mean ± SD)	21.3 ± 13.4	21.2 ± 11.3	0.99 (0.96-1.02)	0.438
Smoking parents[&]				
No smoking parents	30 (17.5%)	47 (14.9%)	1.00	
One smoking parent	102 (59.6%)	197 (62.3%)	0.81 (0.48-1.35)	0.415
Two smoking parents	37 (21.6%)	68 (21.5%)	0.90 (0.48-1.67)	0.737
Missing	2 (1.2%)	4 (1.3%)		
Smoking status partner[#]				
Never smoker	58 (33.9%)	127 (40.2%)	1.00	
Former smoker	65 (38.0%)	123 (38.9%)	1.06 (0.68-1.66)	0.799
Current smoker	35 (20.5%)	46 (14.6%)	1.66 (0.97-2.85)	0.066
Missing	13 (7.6%)	20 (6.3%)		
Nicotine exposure at work[#]				
Never exposed	89 (52.0%)	156 (49.4%)	1.00	
Former exposure	66 (38.6%)	144 (45.6%)	0.79 (0.53-1.18)	0.250
Current exposure	14 (8.2%)	9 (2.8%)	2.75 (1.14-6.63)	0.024
Missing	2 (1.2%)	7 (2.2%)		
Excessive alcohol use[#,^]				
Yes	18 (10.5%)	26 (8.2%)	1.65 (0.84-3.25)	0.147
No	152 (88.9%)	287 (90.8%)		
Missing	1 (0.6%)	3 (0.9%)		
Exposure to hazardous substances at work[#]				
Yes	25 (14.6%)	57 (18.0%)	0.91 (0.48-1.37)	0.423
No	142 (83.0%)	252 (79.7%)		
Missing	4 (2.3%)	4 (2.3%)		
Educational level (continuous)			0.88 (0.79-0.99)	0.037
Educational level				
No / Primary education	44 (25.7%)	65 (20.6%)	1.00	
Secondary education	86 (50.3%)	142 (44.9%)	0.91 (0.57-1.47)	0.708
Higher education	39 (22.8%)	108 (34.2%)	0.55 (0.32-0.95)	0.033
Missing	2 (1.2%)	1 (0.3%)		
Number of comorbidities[#] (mean ± SD)	4.6 ± 2.6	2.5 ± 2.2	1.37 (1.25-1.50)	<0.001
Cardiac disease[#]				
Yes	23 (13.5%)	22 (7.0%)	1.94 (1.03-3.67)	0.041
No	144 (84.2%)	268 (84.8%)		
Missing	4 (2.3%)	26 (8.2%)		

Table S6.1 (continued)

	MC Cases (n=171)	Non-MC Controls (n=316)	Adjusted Odds Ratio (95% CI)*	p-value
Hypertension[#]				
Yes	49 (28.7%)	68 (21.5%)	1.27 (0.82-1.98)	0.292
No	122 (71.3%)	230 (72.8%)		
Missing	0 (0.0%)	18 (5.7%)		
Asthma[#]				
Yes	19 (11.1%)	23 (7.3%)	1.47 (0.77-2.81)	0.247
No	150 (87.7%)	260 (82.3%)		
Missing	2 (1.2%)	33 (10.4%)		
Other Pulmonary disorder[#]				
Yes	25 (14.6%)	26 (8.2%)	1.69 (0.94-3.05)	0.080
No	143 (83.6%)	257 (81.3%)		
Missing	3 (1.8%)	33 (10.4%)		
Diabetes Mellitus[#]				
Yes	6 (3.5%)	16 (5.1%)	0.58 (0.22-1.53)	0.268
No	162 (94.7%)	268 (84.8%)		
Missing	3 (1.8%)	32 (10.1%)		
Gastric disorder[#]				
Yes	20 (11.7%)	18 (5.7%)	1.95 (1.00-3.82)	0.051
No	148 (86.5%)	262 (82.9%)		
Missing	3 (1.8%)	36 (11.4%)		
Renal disease[#]				
Yes	7 (4.1%)	10 (3.2%)	1.10 (0.41-2.98)	0.847
No	163 (95.9%)	270 (85.4%)		
Missing	1 (0.6%)	37 (11.7%)		
Liver disorder[#]				
Yes	11 (6.4%)	5 (1.6%)	3.97 (1.35-11.70)	0.012
No	159 (93.0%)	274 (86.7%)		
Missing	1 (0.6%)	37 (11.7%)		
Hematological disorder[#]				
Yes	23 (13.5%)	24 (7.6%)	1.66 (0.90-3.71)	0.108
No	147 (86.0%)	258 (81.6%)		
Missing	1 (0.6%)	34 (10.8%)		
Cancer[#]				
Yes	8 (4.7%)	23 (7.3%)	0.51 (0.22-1.18)	0.114
No	162 (94.7%)	263 (83.2%)		
Missing	1 (0.6%)	30 (9.5%)		
Depressive mood disorder[#]				
Yes	38 (22.2%)	34 (10.8%)	2.16 (1.29-3.61)	0.003
No	129 (75.4%)	248 (89.2%)		
Missing	4 (2.3%)	34 (10.8%)		
Arthrosis[#]				
Yes	62 (36.3%)	53 (16.8%)	2.53 (1.60-4.00)	<0.001
No	107 (62.6%)	233 (73.7%)		
Missing	2 (1.2%)	30 (9.5%)		
Chronic back pain[#]				
Yes	81 (47.4%)	114 (36.1%)	1.40 (0.95-2.08)	0.089
No	88 (51.5%)	180 (57.0%)		
Missing	2 (1.2%)	22 (7.0%)		

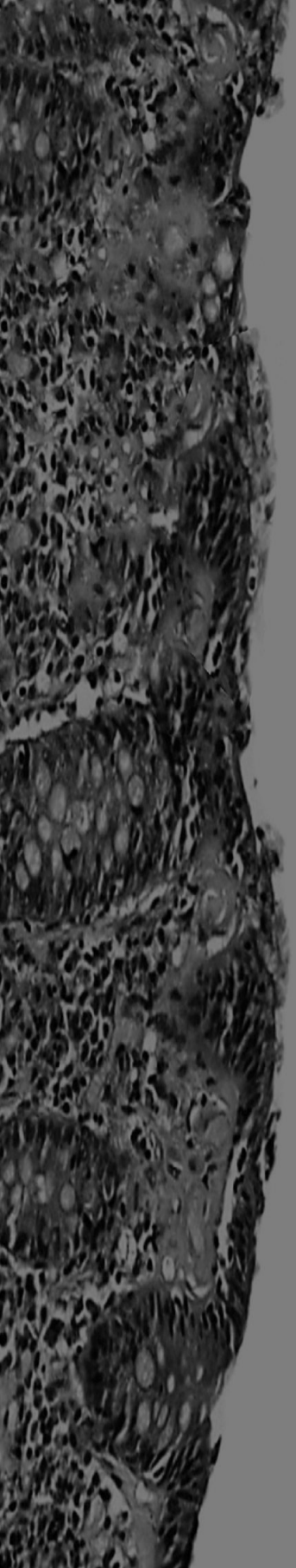
Table S6.1 (continued)

	MC Cases (n=171)	Non-MC Controls (n=316)	Adjusted Odds Ratio (95% CI)*	p-value
Rheumatoid arthritis[#]				
Yes	22 (12.9%)	17 (5.4%)	2.27 (1.16-4.46)	0.017
No	145 (84.8%)	265 (89.2%)		
Missing	4 (2.3%)	24 (10.8%)		
Esophageal disorder[#]				
Yes	8 (4.7%)	2 (0.6%)	7.22 (1.50-34.85)	0.014
No	159 (93.0%)	274 (86.7%)		
Missing	4 (2.3%)	40 (12.7%)		
Thyroid disorder[#]				
Yes	19 (11.1%)	26 (8.2%)	1.24 (0.66-2.33)	0.515
No	149 (87.1%)	261 (82.6%)		
Missing	3 (1.8%)	29 (9.2%)		
Hypercholesterolemia[#]				
Yes	37 (21.6%)	58 (18.4%)	1.10 (0.68-1.77)	0.697
No	131 (76.6%)	234 (74.1%)		
Missing	3 (1.8%)	24 (7.6%)		
Colon carcinoma[#]				
Yes	0 (0.0%)	3 (0.9%)	1.04 (0.17-6.44)[§]	0.969[§]
No	171 (100%)	313 (99.1%)		
Missing	0 (0.0%)	0 (0.0%)		
Celiac disease[#]				
Yes	6 (3.5%)	0 (0.0%)	10.86 (1.29-91.33)[§]	0.028[§]
No	165 (96.5%)	316 (100.0%)		
Missing	0 (0.0%)	0 (0.0%)		
Appendectomy[#]				
Yes	29 (17.0%)	44 (13.9%)	1.21 (0.72-2.03)	0.467
No	142 (83.0%)	272 (86.1%)		
Missing	0 (0.0%)	0 (0.0%)		
Allergies[&]				
Yes	34 (19.9%)	58 (18.4%)	1.13 (0.70-1.83)	0.605
No	135 (78.9%)	251 (79.4%)		
Missing	2 (1.2%)	7 (2.2%)		
Breastfeeding[&]				
Yes	88 (51.5%)	165 (52.2%)	0.76 (0.45-1.28)	0.298
No	35 (20.5%)	54 (17.1%)		
Missing	48 (28.1%)	97 (30.8%)		
Vaccinations[&]				
Yes	142 (83.0%)	288 (91.7%)	0.64 (0.22-1.91)	0.424
No	6 (3.5%)	8 (2.5%)		
Missing	23 (13.5%)	20 (6.3%)		
Frequent antibiotic use (>3x/year)[&]				
Yes				
No	5 (2.9%)	5 (1.6%)	2.19 (0.61-7.81)	0.229
Missing	111 (64.9%)	228 (72.2%)		
	55 (32.2%)	83 (26.3%)		

Table S6.1 (continued)

	MC Cases (n=171)	Non-MC Controls (n=316)	Adjusted Odds Ratio (95% CI)*	p-value
Day care visit[&]				
Yes	24 (14.0%)	36 (11.4%)	1.29 (0.76-2.26)	0.368
No	145 (84.8%)	280 (88.6%)		
Missing	2 (1.2%)	0 (0.0%)		
Siblings				
Yes	166 (97.1%)	302 (95.6%)	1.58 (0.56-4.47)	0.390
No	5 (2.9%)	14 (4.4%)		
Number of siblings (mean ± SD)	4,0 ± 2,8	3,7 ± 2,7	1.02 (0.95 - 1.10)	0.540
Birth order	3,0 ± 2,1	2,9 ± 2,2	1.02 (0.93-1.11)	0.730
Presence of animals at home[&]				
Yes	135 (78.9%)	223 (70.6%)	1.61 (1.03-2.50)	0,037*
No	36 (21.1%)	93 (29.4%)		
Presence of animals at home[&]				
Dog(s) or Cat(s)	128 (74.9%)	196 (62.0%)	1.84 (1.21-2.78)	0.004
Other pets	40 (23.4%)	84 (26.6%)	0.88 (0.56-1.38)	0.577
Live stock	11 (6.4%)	20 (6.3%)	0.99 (0.46-2.13)	0.980
Poultry	23 (13.5%)	53 (16.8%)	0.77 (0.46-1.31)	0.341
Familial occurrence of MC				
Yes	4 (2.3%)	15 (4.7%)	0.65 (0.21-2.02)	0.454
No	83 (48.5%)	215 (68.0%)		
Missing	84 (49.1%)	86 (27.2%)		
Partner with MC				
Yes	1 (0.6%)	7 (2.2%)	1.08 (0.66-1.77)	0.748
No	152 (88.9%)	284 (89.9%)		
Missing / no partner	18 (10.6%)	25 (7.9%)		
Female subjects only	MC Cases (n=138)	Non-MC Controls (n=250)		
Use of oral contraceptives[#]				
Yes	115 (83.3%)	203 (81.2%)	1.47 (0.80-2.68)	0.215
No	22 (15.9%)	47 (18.8%)		
Missing	1 (0.7%)	0 (0.0%)		
At least one pregnancy [#]				
Yes	118 (85.5%)	207 (82.8%)	1.10 (061-2.01)	0.731
No	20 (14.5%)	42 (16.8%)		
Missing	0 (0.0%)	1 (0.4%)		
Number of pregnancies (mean ± SD) [#]	2.3 ± 1.4	1.90 ± 1.2	1.23 (1.04-1.44)	0.015
Age of first menarche in years (mean ± SD)	13,2 ± 1,6	13,3 ± 1.6	0.96 (0.84-1.09)	0.507
Menopausal status[#]				
Premenopausal	42 (30.4%)	90 (36.0%)	1.10 (0.58-2.06)	0.778
Postmenopausal	96 (69.6%)	160 (50.6%)		
Use of hormonal supplements[#]				
Yes	30 (21.7%)	31 (12.6%)	1.89 (1.08-3.29)	0.025
No	108 (78.3%)	216 (86.4%)		
Missing	0 (0.0%)	3 (1.2%)		

MC: Microscopic colitis; SD: standard deviation; 95% CI: 95% Confidence Interval. [#] before the index date. [&] in childhood. [^] excessive alcohol use was defined as ever continuous use of >21 units per week for more than months. * Adjusted for age and gender. [$] Calculated with at least one positive subject in either the case or control group



7

Ambient air quality as risk factor for microscopic colitis – a Geographic Information System (GIS) study

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Submitted

Abstract

Background

Microscopic colitis (MC) is considered a multifactorial disease. However, little is known about the role of environmental factors as ambient air pollution in MC pathophysiology. Given the strong association between MC and smoking, and the overlap in components of cigarette smoke and ambient air pollution, the aim of this study was to explore an independent association between long-term ambient air quality and the risk of MC.

Methods

A case-control study was performed. MC cases in South Limburg, the Netherlands, diagnosed between 2000-2012, were retrieved from the national pathology registry and matched to non-MC controls from the same area based on age (±2 years) and gender. A stable residential address for ≥3 years was required. Residential land use, proximity to major road, and concentrations of air pollution compounds, were determined using a Geographic Information System (GIS). Univariate and multivariable regression analyses were corrected for age, gender and smoking status.

Results

In total, 345 MC cases (78.6% female) and 583 matched controls (77.2% female) were included. In the univariate analyses the percentage of urban green within a 500m buffer and residential proximity to the nearest highway were associated with MC (both $p<0.10$). On the multivariable level only a higher age at diagnosis (OR 1.02, 95%-CI 1.01-1.04) and current smoking at index date (OR 4.30; 95%-CI 3.01-6.14) were significantly associated with MC.

Conclusion

Based on the current findings, ambient air quality does not seem to be an important risk factor for MC, in contrast to the well-known risk factors age and current smoking.

Introduction

Microscopic colitis (MC) is a chronic bowel disorder with frequent watery diarrhoea as primary symptom. Although the aetiology is not clear, MC is considered a multifactorial disease, in which immunological, genetic, microbial, life style and environmental factors play a role.[1] Amongst the latter, smoking and drug exposure have repeatedly been associated with MC.[2-5] Especially (co)exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) seems to increase the risk of MC.[4,5] However, in these studies only 20-40% of MC patients were exposed to these drugs in the year before diagnosis, suggesting that drug-exposure does not explain for all MC cases. Other possible risk factors for MC (*e.g.* hormones, alcohol, socio-economic status) have been addressed in a limited number of studies, but the results were either negative or conflicting.[3,6-8] One study, of small sample size, explored the involvement of microbiota in MC, showing an increased prevalence of *Akkermansia* spp.[9] In other words, further research for possible risk factors is warranted in order to confirm current risk factors and hypotheses on underlying pathophysiological mechanisms, or to find new ones.

Several publications report that smoking is associated with MC.[2,3,10] Tobacco smoke compounds such as particulate matter (PM), nitric oxides and benzene are also present in polluted air. Therefore, ambient air quality (influenced by *e.g.* industrial, agricultural and traffic related emissions in the residential area) may have a contributory effect to the pathogenesis of MC as well. Furthermore, MC peak incidence rates are observed in the older population (>60 years of age), which is also supportive for involvement of environmental factors in the pathogenesis. Considering that people are continuously exposed to polluting sources in the direct environment, such as traffic, industry, urbanized areas or agricultural poisons, ambient air quality might be contributory. Moreover, previous studies reported an association between air pollution and various gastrointestinal conditions, such as gastrointestinal cancer, appendicitis, bowel infections and IBD.[11-15] However, the association with MC has never been reported.

Chemicals present in ambient air can reach the gastrointestinal tract by direct ingestion or via ingestion of pulmonary mucus, which clears inhaled air from particles.[16] Based on *in vitro* and animal studies, air pollutants are postulated to have various compromising intestinal effects, such as DNA damage, disruption of the epithelial barrier and initiation of an innate immune response by activation of immune cells or cell signalling pathways.[17] In individuals with a genetic predisposition for an inflammatory condition, presence of inflammatory cytokines might lead to a more pronounced effect on the epithelial barrier in case of exposure to toxic particles.[18] Beside direct effects on the epithelium, air pollutants induce changes in microbial composition or physiology. In mice models, for instance, PM changed the relative amounts of various bacterial strains and reduced the production of butyrate, impairing epithelial permeability.[19] In addition, some bacteria are able to metabolize ingested particles into toxic metabolites or reactive oxygen species, affecting mucosal barrier function. In general, environmental factors (in)directly induce epigenetic changes during life. In a genetic susceptible host, the sum

of epigenetic changes and environmental influences then may trigger inflammatory activity, not resolving due to the epigenetic changes.[20]
In conclusion, there is circumstantial evidence which suggests that ambient air quality may play a role in MC pathophysiology. Therefore, the aim of this study was to assess the effect of ambient air quality on the risk of MC in a large cohort of MC patients. We hypothesized that exposure to ambient air pollution increases the risk of MC.

Methods

Study population

A case-control study was performed, including MC cases from all three hospitals in the region of South Limburg, the Netherlands. Cases were identified in PALGA, the Dutch nationwide registry of histo- and cytopathology. All cases aged 18 years or older, with a PALGA registered diagnosis of MC, collagenous colitis (CC), lymphocytic colitis (LC), incomplete CC (CCi) or incomplete LC (LCi) between January 2000 and December 2012, were selected. Hereafter, pathology reports and medical charts were reviewed to exclude cases which did not meet the histological or clinical criteria for MC. The haematoxylin-eosin stained sections from diagnostic biopsies were retrieved and revised according to the ESP/EMCG diagnostic criteria[21] by an experienced pathologist (D.G.), in order to verify the diagnosis. The pathologist was blinded for any clinical symptoms and the previously established diagnosis.
Non-MC controls were retrieved from the general South Limburg population. Study information letters were sent to households within the study area. Those were randomly selected based on postal code. Per household, informed consent was asked to a maximum of four subjects above 18 years of age. Of this control population (n=1,611), two non-MC controls were matched to each MC case, based on gender and year of birth (± 2 years). If a control was matched, any other member from the same household was excluded for further matching. Frequency matching was applied to ensure that the control population had the same distribution over all municipalities as the background population of South Limburg.
The index date of the cases was defined as the date of histological diagnosis. Non-MC controls were assigned the same index date as their matched case.

Residential data

For each subject, historical and current residential data were retrieved from the national civil registration system (in Dutch: Basisregistratie Persoonsgegevens, https://www.rvig.nl/brp), kept by the Dutch Ministry of Internal Affairs. For each subject, the residential address at index date was recorded. Only subjects with a stable residential address for at least three years before the index date, were considered

suitable for inclusion. Residential addresses were geocoded, based on the data provided by the 'Basisregistratie Adressen en Gebouwen', a database maintained by the national Cadastre survey (https://www.kadaster.nl/bag).

Markers for ambient air quality

Both direct and indirect markers for ambient air quality were included in the study, *i.e.* main air pollution components, land use data, population density, urbanity, proximity to major roads, and total road length in the residential area. Based on the geocoded residential addresses, individual exposure to the various variables was assessed, applying geographic information system (GIS) functions (ArcGIS 9.3. Esri, Redlands, CA, USA). Figure 7.1 (panel C-F) gives an impression on the spatial distribution of some of the included markers for ambient air quality.

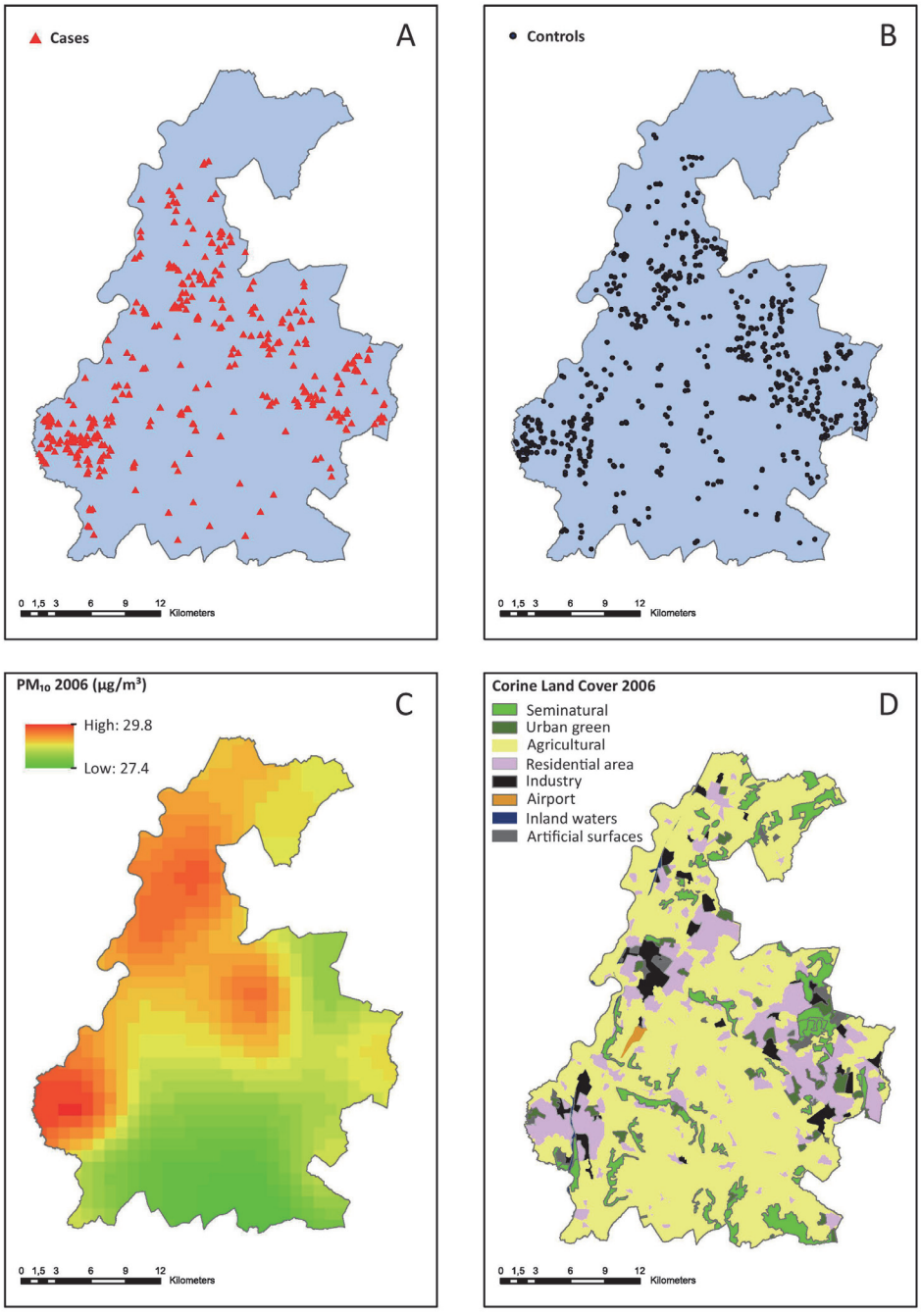
Data on regional concentrations of the most common air pollutants according to the World Health Organisation, *i.e.* particulate matter ≤10µm (PM_{10}), nitric dioxide (NO_2), ozone (O_3), sulphur dioxide (SO_2) and benzene (C_6H_6), were obtained from the National Institute for Public Health (RIVM) of the Dutch Ministry of Health, Welfare and Sports (http://www.lml.rivm.nl). Air pollutant concentrations (µg/m^3) were measured ad 60 fixed monitoring stations in the Netherlands. Measured concentrations, added with local and foreign data, were applied in a dispersion model to calculate local concentrations on a 5x5km grid and interpolated to a 1x1km grid.[22] A margin of uncertainty of 15% around the interpolated data was calculated by the RIVM. Data were available for each year between 2000 and 2012. In the study analyses, SO_2 and benzene were not included, as rational concentrations were too low to expect any health risks.[22]

Land use data were retrieved from CORINE Land Cover, a cartographic database (scale 1:100,000) coordinated by the European Environment Agency (EEA).[23] Each 100x100m was assigned to its major type of land use, being residential (CORINE class 1.1), industrial (class 1.2.1), urban green (class 1.4.1), agricultural (class 2) or natural areas (class 3). Data were available for the years 2000, 2006, and 2012. Within 100m, 500m and 2500m radius buffers from each residential address, the percentages of the various types of land use were determined. Data were not restricted to national borders.

Traffic intensity was reflected by a) the total road length within 100m, 200m, 500m radius buffers from the residential address and b) the residential proximity to the nearest highway or major road (defined as national roads with >10.000 vehicles per day). The proximity was calculated based on digital street maps and was expressed as a logarithmic function.

Population density (number of inhabitants per km^2) and urbanization rate (number of addresses per km^2) were derived from demographic data of Statistics Netherlands (CBS) (https://www.cbs.nl). Data were available per neighbourhood (an area often smaller than 1 km^2, *e.g.* a small village or a part of a larger village or city district.) for the years 2004, 2009, and 2012.

For all analyses data most close to the concerning index date were applied.


Cases
A
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PM10 2006 (µg/m3)
C
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Low: 27.4
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Corine Land Cover 2006
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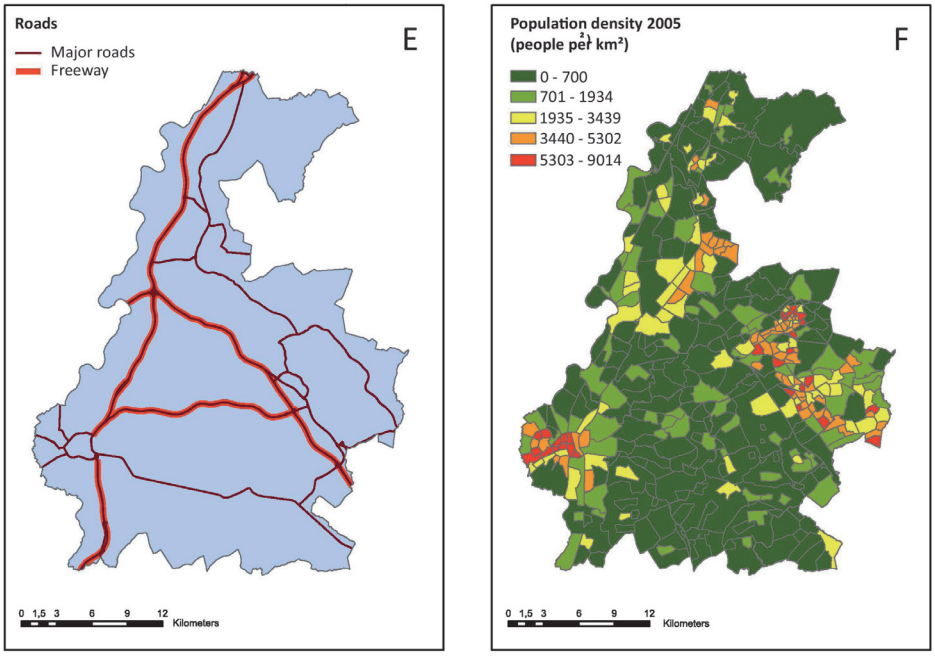

Figure 7.1 Panel A and B show the distribution of the included cases and controls in South Limburg. Panels C-F visualize the spatial distribution of particulate matter (PM) (C), land use (D), major roads (E) and population density (F) in South Limburg, for the year 2006. These panels give an impression of the source data and do not represent the complete data, as multiannual data were used for the analyses.

Statistical analysis

Continuous variables were expressed as means with standard deviation (SD). With regard to the patient characteristics, statistical significant differences between cases and controls were calculated using an independent Student's T-test for continuous variables or a Chi^2-test for categorical variables. For all other variables, univariate logistic regression analysis was applied to calculate odds ratios (OR) with 95%-Confidence Intervals (95%-CI). All variables with a p-value <0.10 in the univariate analyses were included in the multivariable logistic regression analysis. Both univariate and multivariable models were corrected for the confounders age, gender and smoking status at index date. In the case population, smoking status at index date was obtained by scrutinizing patient's medical files. Controls were asked for their smoking status (including year of cessation, if applicable) via a brief questionnaire added to the informed consent form. Missing data on smoking status were imputed by applying a fully conditional specification method, which randomly imputes missing data based on the other results. Presence of multicollinearity between selected variables was assessed by

requesting collinearity diagnostics in the statistical program. Statistical significance was determined as $p<0.05$.
For all analyses, CCi and LCi cases were included in the CC and LC group, respectively. Furthermore, in case biopsy specimens could not be revised, the diagnosed subtype as recorded in the pathology report was acquired, provided that the diagnosis was set with a high or moderate likelihood (24). A sensitivity analysis was performed, excluding CCi and LCi cases and cases without a biopsy proven diagnosis.
All analyses were conducted with IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

Ethical Considerations

This study was performed according to the revised version of the declaration of Helsinki and approved by the medical ethical committee of Maastricht University Medical Centre^{+} (NL44127.068.13 and NL31636.068.10). Written informed consent was obtained from all participants.

Results

Study population

In total, 430 MC cases were selected from the three participating centres, of which 77 cases were excluded for the following reasons: they were living outside the designated study area at index date (n=5), they lived <3 years on the residential address since at index date (n=60), or they were included in two centres at the same time (n=2). In addition, 18 cases were excluded because the diagnosis could not be confirmed upon biopsy revision.
The remaining 345 cases (271 females, 78.6%) consisted of 108 (31.1%) CC, 176 (51.0%) LC, 6 CCi (1.7%) and 28 (8.1%) LCi cases. In 27/345 cases (7.8%, 15 CC and 12 LC), no biopsy material was available for revision (Table 7.1). As all of them had a high likelihood of a positive MC diagnosis based on the pathology report, they were included for further analyses.
All 345 cases were matched to 583 non-MC controls (450 females, 77.2%). The spatial distribution of the included subjects is visualized in Figure 7.1 (panel A and B). A 1:2 matching ratio was achieved in 69.0% of cases. As presented in Table 7.1, the mean age at index date was 63.3 ± 13.0 years for the cases and 61.1 ± 12.4 years for the controls ($p=0.01$). On average, the duration of an unchanged residential address before the index date was 21.3 ± 12.8 and 21.3 ± 12.1 years ($p=0.96$) for cases and controls, respectively.
In total, the smoking status at index date was missing in 13.0% of the cases and 4.3% of the controls. After imputation, the proportion of current smokers was 43.8% in the case population and 15.3% in the control population ($p<0.01$) (Table 7.1).

Table 7.1 Baseline characteristics

	Cases (n=345)	Controls (n=583)	p-value
Female, n (%)	271 (78.6%)	450 (77.2%)	0.66
Age at index date, mean years ± SD	63.3 ± 13.0	61.2 ± 12.4	0.01
Duration of stable address before index date, mean years ± SD	21.3 ± 12.8	21.3 ± 12.1	0.96
Type of MC – PA revision			
CC, n (%)	108 (31.3%)		
LC, n (%)	176 (51.0%)		
CCi, n (%)	6 (1.7%)		
LCi, n (%)	28 (8.1%)		
Unrevised, n (%)*			
CC, n (%)	15 (4.3%)		
LC, n (%)	12 (3.5%)		
Smoking status at index date			
Current, n (%)	151 (43.8%)	89 (15.3%)	<0.01
Former, n (%)	81 (23.5%)	236 (40.5%)	<0.01
Never, n (%)	113 (32.8%)	258 (44.3%)	<0.01

CC: Collagenous colitis; LC: lymphocytic colitis; CCi: incomplete collagenous colitis; LCi: incomplete lymphocytic colitis. * Diagnosis was based on the conclusion provided in the pathology report

Environmental factors

Univariate analyses

Data on the various measured (proxy) markers for ambient air quality were presented in Table 7.2. Only the percentage of urban green within a 500m radius buffer around the residential address (OR 0.20; 95%-CI 0.03-1.27) and residential proximity to the nearest highway (OR 0.93; 95%-CI 0.87-1.00) were statistically different between cases and controls on a p<0.10 level. No statistical differences were observed between cases and controls regarding the percentage of industrial, residential, agricultural, natural or urban green area, total road length around the residential address, population density, address density, or major air pollution compounds (Table 7.2).

Multivariable analysis

Next to the percentage of urban green within a 500m radius buffer and residential proximity to the nearest highway; gender, age and smoking status at index date were included in the multivariable model. Based on this model, a higher age at diagnosis (OR 1.02, 95%-CI 1.01-1.04) and current smoking at index date (OR 4.30; 95%-CI 3.01-6.14) were significantly associated with a higher risk of MC (Table 7.3).

Table 7.2 Univariate analyses of direct and indirect markers for ambient air pollution

	Cases (n=345)	Controls (n=583)	Crude Odds Ratio (95%-CI)	Adjusted Odds Ratio (95%-CI)*	p-value
Air pollution components (μg/m^3)					
PM_{10}, mean ± SD	27.36 ± 3.02	27.17 ± 2.94	1.02 (0.98-1.06)	1.02 (0.98-1.07)	0.35
O_3, mean ± SD	36.96 ± 3.28	37.04 ± 3.23	0.96 (0.92-1.00)	0.97 (0.93-1.01)	
NO_2, mean ± SD	25.15 ± 3.71	24.77 ± 3.55	1.03 (0.99-1.01)	1.03 (0.99-1.07)	0.17
Land use					
Industrial area, mean %					
100m buffer	1.31%	1.29%	1.02 (0.25-4.13)	1.58 (0.36-6.90)	0.55
500m buffer	2.89%	3.59%	0.48 (0.12-1.94)	0.74 (0.16-3.36)	0.74
2500m buffer	8.37%	7.88%	2.88 (0.42-19.86)	2.19 (0.28-17.14)	0.46
Residential area, mean %					
100m buffer	83.73%	82.01%	1.81 (0.78-1.80)	1.13 (0.72-1.76)	0.60
500m buffer	66.92%	63.87%	1.50 (0.92-2.44)	1.38 (0.82-2.33)	0.22
2500m buffer	33.83%	32.72%	1.47 (0.67-3.22)	1.20 (0.52-2.77)	0.67
Urban green, mean %					
100m buffer	0.79%	1.65%	0.31 (0.06-1.78)	0.32 (0.05-1.99)	0.22
500m buffer	2.46%	3.37%	0.24 (0.04-1.37)	0.20 (0.03-1.27)	0.09
2500m buffer	3.59%	3.66%	0.56 (0.01-25.79)	0.25 (0.00-15.17)	0.51
Nature, mean %					
100m buffer	0.98%	0.99%	0.99 (0.18-5.38)	1.53 (0.25-9.26)	0.65
500m buffer	2.59%	2.04%	2.54 (0.46-14.12)	2.40 (0.39-14.95)	0.35
2500m buffer	5.82%	6.11%	0.45 (0.05-4.10)	0.72 (0.07-7.50)	0.78
Agricultural area, mean %					
100m buffer	13.11%	13.77%	0.92 (0.59-1.46)	0.91 (0.56-1.48)	0.69
500m buffer	23.98%	25.81%	0.79 (0.49-1.28)	0.84 (0.51-1.41)	0.52
2500m buffer	44.75%	46.31%	0.73 (0.40-1.33)	0.84 (0.44-1.58)	0.58
Roads					
Total road length (km), mean ± SD					
100m buffer	0.93 ± 0.37	0.89 ± 0.36	1.30 (0.91-1.88)	1.06 (0.71-1.56)	0.78
200m buffer	3.55 ± 1.23	3.38 ± 1.16	1.13 (1.01-1.26)	1.06 (0.94-1.19)	0.35
500m buffer	18.76 ±6.29	18.08 ± 6.05	1.02 (1.00-1.04)	1.01 (0.99-1.03)	0.42
Residential proximity to the nearest major road (km), mean ± SD	0.81 ± 0.71	0.83 ± 0.70	0.94 (0.78-1.14)	0.94 (0.77-1.16)	0.57
Residential proximity to the nearest highway (km), mean ± SD	2.52 ± 1.97	2.83 ± 2.09	0.93 (0.87-0.99)	0.93 (0.87-1.00)	0.06
Demography					
Number of inhabitants*1,000 per km^2, mean ± SD	2.87 ± 1.92	2.79 ± 1.86	1.02 (0.95-1.10)	1.01 (0.94-1.09)	0.80
Number of addresses*100 per km^2, mean ± SD	1.28 ± 0.81	1.18 ± 0.69	1.02 (1.00-1.04)	1.02 (1.00-1.04)	0.11

PM_{10}: Particulate matter <10μm, 95%-CI: 95% Confidence Interval; * Adjusted for gender, year of birth and smoking status

Table 7.3 Results of the multivariable analysis

	Odds Ratio (95%-CI)
Female Gender	1.03 (0.73-1.47)
Age at index date (years)	1.02 (1.01-1.04)
Smoking status at index date	
Never smoker	1.00
Former smoker	0.78 (0.55-1.10)
Current smoker	4.30 (3.01-6.14)
Percentage of urban green (500m buffer)	0.19 (0.03-1.20)
Residential proximity to the nearest highway (km) (logarithm)	0.78 (0.58-1.06)

95%-CI: 95% Confidence Interval

Sensitivity analysis

All analyses were repeated after exclusion of CCi/LCi cases and cases with unrevised biopsies. In total, 284 cases and 481 matched controls were included. The general outcomes of the univariate analyses remained unchanged, except for the fact that urban green within a 500m radius buffer was not statistically different anymore in the univariate analysis. The results of the multivariable analyses, including age, gender, smoking status and residential proximity to the nearest highway, remained unchanged, with a significant association for a higher age (OR 1.02; 95%-CI 1.01-1.04) and current smoking (OR 4.20; 95%-CI 2.83-6.23).

Discussion

This is the first study to explore the role of ambient air quality on the risk of MC. Although various direct and indirect markers for ambient air quality were studied, none of them were significantly associated with an increased risk of MC on a multivariable level. Though, the previously reported risk factors age and current smoking were confirmed.

Ambient air is a heterogeneous composition of gases, particles and volatile organic compounds (VOCs) (*e.g.* benzene). Especially PM_{10} and NO_2, emitted by both natural and anthropogenic sources (*e.g.* industry, traffic, agriculture), have a negative impact on general health by increasing the risk of mortality, hospital admissions and pulmonary, cardiovascular or gastrointestinal disorders.[17,25] However, the results of the present study showed that the primary components and proxy markers for air pollution (*e.g.* PM_{10}, high industrial area, residential proximity to roads) were not associated with MC on the univariate and multivariable level, which makes a major role for ambient air quality in MC pathophysiology unlikely. However, a possible contributory effect should not be discarded completely. Although the level of exposure to ambient air pollution was not different between MC cases and controls, the effect of this exposure might still be different between the two populations, for instance due to (genetic) susceptibility. As an example, in inflammatory bowel disease the *PTPN2*-gene is only associated with Crohn's disease in a smoking population,[26] which supports the hypothesis that specific genetic variants are required for specific environmental factors to contribute to disease development.

In contrast to most (proxy) markers for ambient air quality, current smoking was found to be an incontestable risk factor for MC, which is in line with various recent studies.[2,3,10,27] Considering the fact that there is overlap in the composition of cigarette smoke and polluted air, the results of this study might be an argument that other compounds of cigarette smoke, generally not present in polluted air (*e.g.* nicotine) do account for the strong associations. However, it should be noted that the level of

exposure to toxic gases and particles via cigarette smoke is considerably higher and more frequent in comparison to ambient air pollution.

The strength of this study resides in the ability to link the subject's residential address at index date and to link it to outcome variables from the same area and time period, which increased the validity of the results. Although no eminent role for ambient air quality was observed in this study, similar approaches linking ambient air quality to health outcomes were able to detect significant associations.[15,28] In addition, the case population in the current study was well-defined, relatively large, and with biopsy proven in 92% of the cases. Although the control population was two years younger on average, we do not think this had major impact on the study outcome. Last, analyses have been corrected for a major MC risk factor, *i.e.* smoking status at index date. Despite 4-13% of data was missing, residual confounding was reduced by imputation of these missing data.

Although the conclusion of our study was that exposure to ambient air quality does not affect the risk of MC, some limitations can be identified that may have influenced this result. First, the size of the study area may have been too small. Consequently, there might have been not enough variation between the studied variables, or the source data were not detailed enough (*e.g.* data per km^2). Second, the sample size may have been too small to detect small variations in the exposure variables. Third, the residential address is often not the location were people spend most of their day (work, traveling, time spend in traffic). Therefore, exposure to environmental factors on the primary residential address may not be most representative for an individual's level of exposure to air pollution. Fourth, the source data applied in this study were not available for each index year. Although most recent data relative to the index date were applied, and three-year average concentrations for air pollution compounds were calculated, small annual variations in the exposure variables might have influenced the outcomes. Finally, it should be noted that data on drug exposure at index date (*e.g.* NSAIDs or PPIs) were not available.

In conclusion, this was the first study to explore an association between ambient air quality and the risk of MC. Based on the results of this study, ambient air quality is not a major contributing factor to the risk of MC. In order to confirm the current findings, additional research studying the role of environmental pollution in large, well-defined populations from other countries would be of value.

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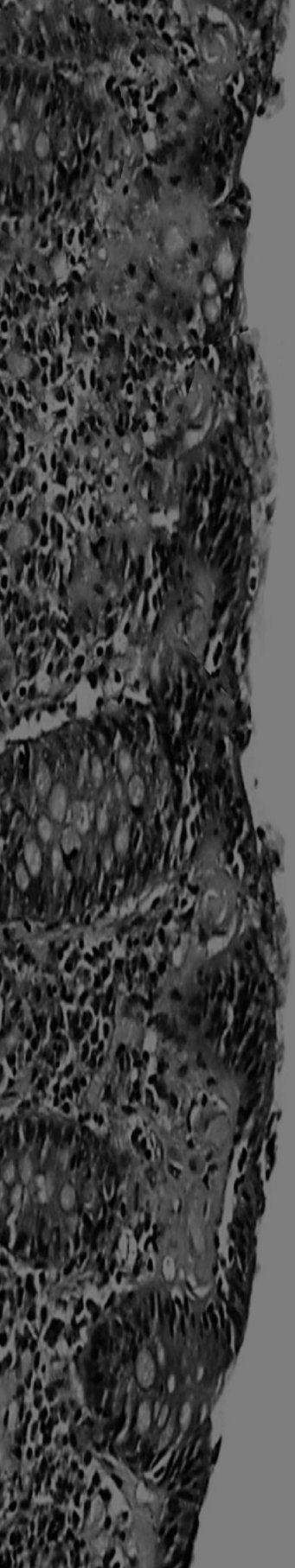

8

PRO-MC collaboration: establishment of a prospective registry for microscopic colitis in Europe - A UEG link award project

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Abstract

Background

Microscopic colitis (MC) is an invalidating inflammatory disorder of the colon with chronic watery diarrhea as main symptom. To date, the disease course of microscopic colitis is largely unpredictable. Small, retrospective studies point towards an intermittent or chronic disease course, with low rates of spontaneous remission. Prospective studies on the disease course of MC are lacking and valid markers to predict the disease course at diagnosis have not yet been identified. To adequately assess the disease course of MC, a large and preferentially non-selected patient cohort is required. Considering the variable incidence rates of MC across Europe, international collaboration is indispensable. Therefore, the aim of the PRO-MC Collaboration is to systematically and prospectively collect data on the long-term disease course of MC and to improve the knowledge on MC across Europe.

Methods

By a systematic expert panel session and selection process, the outcomes of interest were formulated, i.e. the persistence, respectively recurrence of diarrhea and health-related quality of life of incident MC cases following diagnosis, the outcome of the treatments applied, and the characteristics of budesonide non-responders.

Hereafter, a consensus on a follow-up strategy was reached and case record forms (CRFs) were drafted. Based on the CRFs, a web-based registry was built. Since pathology assessment is crucial for the diagnosis of MC, a slide kit presenting the diagnostic criteria and recommended stains was developed in collaboration with members of the European Society of Pathology (ESP), in order to increase the awareness of MC among pathologists and to improve data validity. All European MC centres can participate in the project and all new cases of MC in the participating centers are eligible for inclusion.

Results

For follow-up, a strategy with fixed visits was preferred. Patient data will be collected at diagnosis and at follow-up. Follow-up visits are scheduled 3, 6, and 12 months after diagnosis, yearly thereafter, and in case of clinical relapse. The CRFs include variables on demographics, symptoms, medical history, drug use, endoscopy and histology, disease activity, quality of life, comorbidity, treatment, and complications. All data will be recorded in a fully customized, web-based data registry. Disease activity and quality of life scores will be documented using a one-week defecation diary and the Short Health Scale questionnaire.

Conclusion

The PRO-MC Collaboration, initiated with support of the UEG LINK Award, used a standardized approach to develop case record forms and a follow-up strategy, incorporated in an international, web-based registry for MC patients. The registry will generate novel insight into the long-term disease course of MC, and could possibly identify markers to predict the disease course and treatment outcome. The systematic, Europe-wide data collection will enhance the applicability of the project results and increase the awareness for the disease.

Introduction

Microscopic colitis (MC) is an intestinal disorder characterized by chronic, watery, non-bloody diarrhea. Although the macroscopic appearance of the colonic mucosa is generally normal, typical inflammatory changes are present at histological examination. MC includes two main subtypes, i.e. collagenous colitis (CC) and lymphocytic colitis (LC) and predominantly affects females and persons above 65 years of age.[1-5] The reported incidence rates of MC in Europe differ between countries. In Sweden, mean annual incidence rates up to 5.4 and 4.5 per 100,000 person were reported for CC and LC, respectively,[6,7] while the Dutch are only 1.8 and 1.3 per 100,000 person years.[8] Varying awareness for the disease is a likely explanation for the observed variations.

Based on observational data, MC is considered as a chronic, benign condition that tends to follow a continuous or intermittent disease course with complete resolution of symptoms in about 50-65% of patients after 3-4 years. A short-term (<3 months) spontaneous remission is expected in less than 5%.[9-13] However, a patient's exact disease course at diagnosis is still largely unpredictable because prospective data are lacking. Better understanding of the disease course will generate more insight in the number of patients that requires treatment, optimal treatment time and risk factors for symptom relapse.

Oral budesonide is the treatment of first choice in MC, inducing clinical remission in more than 80% of patients.[14] However, 60-80% of them experience a relapse of symptoms after cessation of treatment[15-17] and 10-20% of those will turn out to be non-responder to oral budesonide.[17] Currently, the characteristics of budesonide non-responders are unknown and (non-invasive) markers to monitor or predict treatment outcome are not available. Apart from oral budesonide, there are no alternative evidence-based treatment options.

In short, prospective studies with systematic data registration are needed to assess: the disease course, treatment outcomes, characteristics of therapy refractory cases, and non-invasive markers for disease course prediction or therapy response. To enable this, systematic registration of incident cases and eventually collection of biomaterials is of relevance. However, this requires a large cohort of non-selected and well described patients. Considering the varying incidence rates, international collaboration is indispensable. Therefore, the aim of the PRO-MC Collaboration is to initiate a European, web-based registry with prospective collection of demographic, clinical and histopathological data.

Development of the PRO-MC Collaboration registry

To enable the initiation of the PRO-MC Collaboration, financial support was applied for and obtained via an UEG Link Award 2014, written by the European Microscopic Colitis Group (EMCG) and supported by the Spanish and Swedish National Societies.

Project design

At project initiation, a project steering group was formed, consisting of 6 persons from 4 different countries (*i.e.* Denmark, Germany, the Netherlands and Sweden). Hereafter, an inventory was sent to dedicated MC researchers, to explore the scientific needs for MC research. Based on the responses, research questions were distilled, especially taking relevancy and feasibility into account. During steering group meetings, variables to be recorded were selected and defined, a standardized follow-up strategy was designed and in- and exclusion criteria were set. It was decided to collect study data at patient inclusion, at 3, 6, and 12 months after inclusion and yearly thereafter (Figure 8.1).
Primary study outcomes were defined as: the long-term disease course of MC, the treatment regimens applied for MC in daily clinic and their outcomes, the characteristics of budesonide non-responders, and the quality of life of MC patients.[18]

Study variables

At each visit, data regarding patient characteristics, disease phenotype, disease activity, treatment response, disease course, medication use, health related quality of life and histopathology will be recorded. Data will be derived via direct patient contact and from patient's medical charts. Health related quality of life and disease activity will be assessed by applying the Short Health Scale questionnaire and a one week defecation diary, respectively.

Study materials

Based on the variables to record and the defined follow-up strategy, all electronic case record forms (eCRFs) were created. These formed the base for the codebook, which provided the IT-company a detailed prescription of all the variables included in the web-based registry (*e.g.* variable name, label, follow-up visit, fill-out options etc.). Furthermore, all study documents required to inform participating centers, to inform eligible patients, or to obtain local ethical permission, were drafted by the Steering Group and made publically available on the EMCG homepage (www.emcg-ibd.eu).

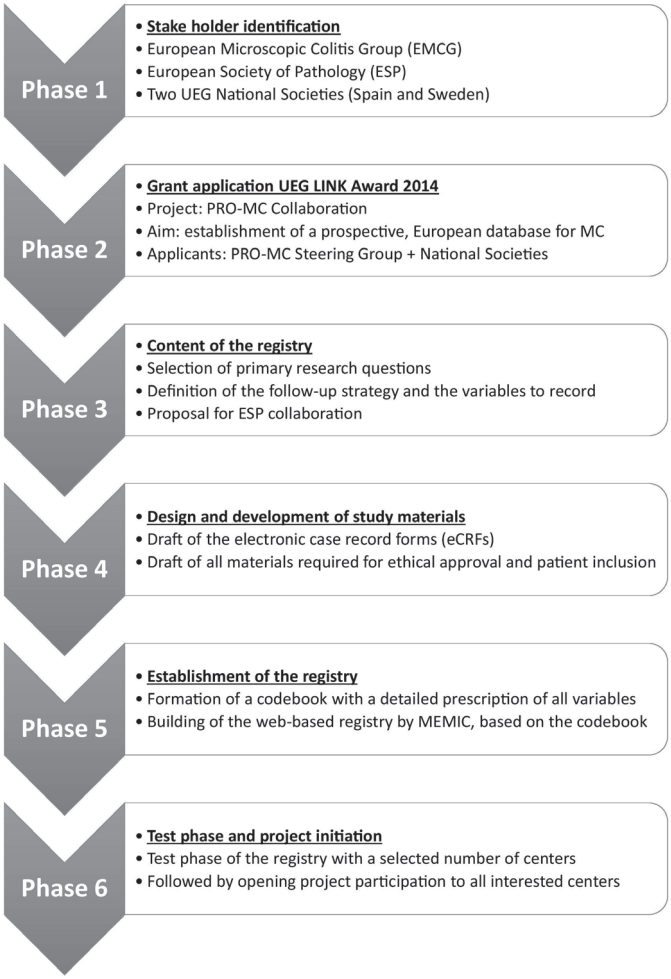

Figure 8.1 Flowchart of the subsequent steps taken in the development of the PRO-MC registry

Technical development

A web-based and user-friendly registry program, applicable in daily clinical care, was considered essential and data safety had to be guaranteed. Eventually, MEMIC (a Maastricht University affiliated IT-company) built the online registry, based on the codebook provided. After a short test period with a limited number of participants, the registry was opened for general participation in May, 2016.

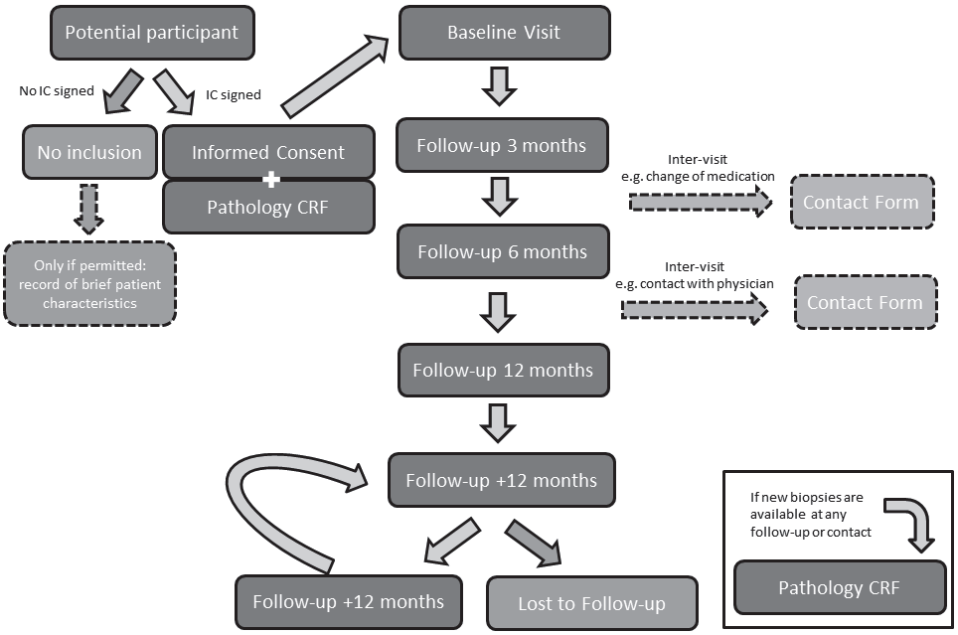

Figure 8.2 Flowchart of the inclusion and subsequent study visits of the PRO-MC Collaboration

Study population

Any patient, 18 years or older, attending the gastroenterology outpatient clinic or endoscopy unit with a new diagnosis of MC (CC, LC, or incomplete MC (MCi)), is eligible for participation in this study. Patients have to be diagnosed according to the internationally accepted criteria for MC *i.e.* chronic watery, non-bloody diarrhea, a (near to) normal colonoscopy, and typical histological abnormalities as defined in recent guidelines.[19,20]

ESP collaboration

Collaboration was sought with the European Society of Pathology (ESP), in order to initiate pathology sub studies, parallel to the PRO-MC registry. Hereto, a digital training tool (slide kit) was developed, including images of MC histology slides, to further inform pathologists on the histologic characteristics of the disease and to increase the diagnostic validity of included cases. The slide kit was made available on the EMG

homepage (www.emcg-ibd.eu). Furthermore, the registered data on MC histology will be used to assess correlations between histology and disease course. Last, inter-observer studies are planned.

Conclusion

The PRO-MC Collaboration incorporates predefined variables and a standardized follow-up strategy in an international, web-based registry for MC patients. The registry aims to generate novel insight in the long-term disease course of MC and to identify markers for prediction of disease course and treatment outcome. The systematic, European data collection will enhance the applicability of the project results and increase the awareness for the disease.

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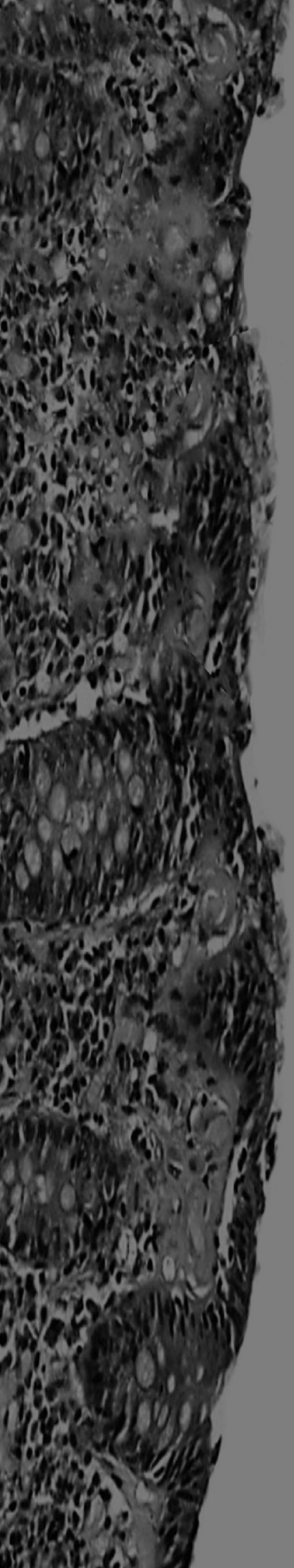

9

General discussion

General discussion

Microscopic colitis (MC) is a chronic inflammatory disorder of the colon. Worldwide the incidence is increasing.[1] In Chapter 2 we have shown that also in the Netherlands, the MC incidence rates increase annually. Beside an increasing awareness for the disease and more performed colonoscopies, the ageing population is also considered to contribute to rising incidence rates. However, changes in risk factor exposure cannot be excluded. Unfortunately, our current knowledge on MC risk factors is relatively limited and the exact aetiology of MC is still unclear. It has been observed that age, gender, smoking, drug use and comorbidities are associated with an increased risk of MC. Considering the rising incidence, it is of relevance that we are aware of risk factors for MC, as they might help to identify and treat MC patients. Therefore, the aims of this thesis were to critically analyse whether previously described risk factors for MC are also present in the Dutch population and to explore novel risk factors, with attention for possible underlying mechanisms.

Pathophysiology

Although the exact pathophysiology of MC remains unclear, some possible mechanisms underlying MC have been investigated, including altered immune function and host genetics (Figure 9.1). In both patients with lymphocytic colitis (LC) and collagenous colitis (CC) increased numbers of $CD3^+$, $CD8^+$, and $Foxp3^+$ (regulatory) T-cells cells were found in colonic biopsies.[2] Increased numbers of $Foxp3^+$ T-cells cells have also been reported in various autoimmune disorders, which often co-occur with MC. In MC, the increased levels of regulatory T-cells are thought to exhibit a mitigating effect on the colonic inflammation, which is supported by increased concentrations of IL-10.[2] However, the evidence is still limited and more detailed analyses on the role of both the innate and adaptive immune system in MC are warranted. A few studies addressed the involvement of host genetics in MC. The most consistent finding was an increased prevalence of HLA-gene polymorphisms, and more specifically, the celiac disease-related HLA-DQ2 and DQ1,3 haplotypes.[3-6] Family and twin studies, as well as genome wide association studies are currently lacking. Nevertheless, more insight in the genetic susceptibility of MC is warranted.

Clinical observations indicate that diversion of the faecal stream is able to resolve MC symptoms and inflammation.[7,8] Based on these findings, it was hypothesized that luminal contents elicit an immunological response, inducing mucosal inflammation thereby leading to clinical symptoms of diarrhoea. Following this line and considering the impact of gastric acid inhibiting PPIs on MC a (Chapter 4+5), it will be interesting to explore the role of intestinal microbiota in MC. So far, only one small-sample study has addressed the microbial composition in MC. The authors reported on a decreased

number of *Akkermansia spp.* in patients with MC versus controls.[9] No difference in the relative abundance was observed for other taxa, such as *Bacteroides,* Lactobacilli, or Bifidobacteria. Further larger scale studies are needed to obtain more detailed information on the microbiota composition and activity of MC patients, differences therein between CC and LC patients, and their relation to therapy success or failure.

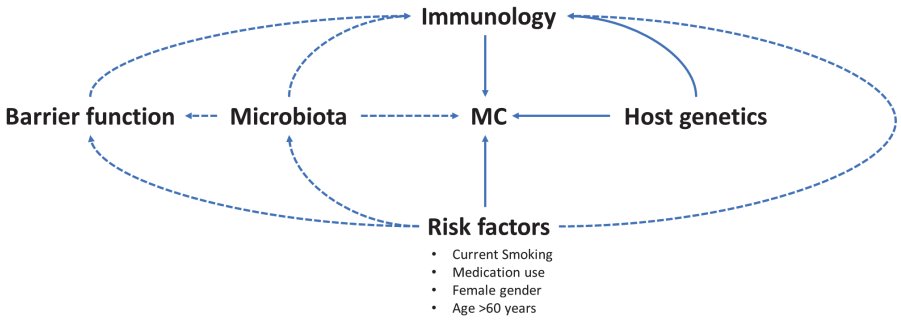

Figure 9.1 Current scientific insights in the pathophysiology of Microscopic Colitis (MC). Based on the current literature, specific genetic factors (e.g. celiac disease related HLA-polymorphisms) and immunologic factors (e.g. increased numbers of CD3+, CD8+, and Foxp3+ (regulatory) T-cells cells and increased mucosal mRNA expression of Th1 and Th17 related cytokines) are presumed to be involved in MC pathogenesis. The current thesis confirms results from literature that risk factors such as cigarette smoking and NSAID/PPI use are strongly associated with MC. Furthermore, circumstantial evidence was provided that these drugs may possibly lead to changes in barrier function and/or microbiota composition, which might induce specific immunological reactions leading to MC symptoms.
Solid lines: pathways for which sufficient evidence is present in literature. *Dashed lines*: pathways, hypothesized to be involved in MC pathophysiology based on limited or circumstantial evidence.

Epidemiological and clinical studies on risk factors for MC are of value to find new leads for underlying pathophysiological mechanisms. Two risk factors that have repeatedly been associated with MC are smoking and drug exposure. Both have also been confirmed as strong MC risk factors in our studies (Chapters 4-7). Smoking has a pronounced negative impact on MC clinical symptoms and on disease course. This is based on the finding that smokers are about 10 years younger at time of diagnosis, have more watery stools at baseline and have a lower chance to achieve clinical remission after treatment.[10] In line with Crohn's disease, the underlying mechanism how cigarette smoke induces/exacerbates MC remains to be elucidated.[11] One option is that nicotine reduces colonic blood flow, thereby negatively affecting microcirculation in the mucosa, which subsequently leads to local ischemia and inflammation.[12]
With regard to drug exposure, strongest associations have been observed for non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs). However, recent studies and case-reports did not explore possible mechanisms by which these

drugs might induce MC. Therefore, in Chapter 4 and 5 of this thesis, we focussed on the exposure characteristics of MC patients compared to controls and on the effect of the associated drugs on epithelial barrier function. Based on the results, especially current, recent and concomitant continuous use of NSAIDs and PPIs, for a period of 4-12 months before diagnosis was associated with an increased risk of MC. These more detailed pharmaco-epidemiological analyses help to hypothesize on possible underlying pathophysiological mechanisms of drug-induced MC. For instance, the associations with current, but also recent use might indicate that the underlying mechanism needs a certain time to develop before symptoms appear, making an idiosyncratic drug reaction less likely. With regard to NSAIDs, the MC risk of cyclooxygenase-2 specific versus non-specific NSAIDs was not different, implying that prostaglandin-related mechanisms are not likely to be involved. For PPI users and for users of H_2-receptor antagonists, the associations with MC point towards a possible role for acid suppression related intestinal changes such as bacterial overgrowth and/or altered microbiota composition and activity. Considering the high MC risk for concomitant NSAID and PPI exposure observed in our studies, a 'two hit model' has to be considered. This would imply that the effects of one drug class might be aggravated or triggered in the presence of the other, leading to mucosal changes or inflammation.

A possible mechanism often hypothesized for both NSAIDs and PPIs to cause MC, is by comprising the epithelial barrier function. Two studies using fresh colonic tissue mounted in Ussing chambers observed that a decreased trans epithelial electrical resistance (TEER) is present in CC patients compared to non-MC controls, possible related to a decreased expression of tight junction proteins claudin-4 and occludin.[13,14] Furthermore, an increased trans mucosal uptake of non-pathogenic bacteria in CC patients compared to non-MC controls was found. Remarkably, this remained present after short-term clinical remission, possibly indicating a permanent disturbance of the colonic epithelial barrier in CC.[15] We also, have evaluated epithelial barrier function in CC (Chapter 5). We analysed the effect of NSAIDs and PPIs on colonic epithelial barrier *ex vivo*, using biopsies of CC patients in Ussing chamber experiments. Biopsies were exposed to the drugs of interest from the basolateral side (*i.e.* mimicking the systemic compartment). Because of the rapid absorption of these drugs in the upper gastrointestinal tract, a direct luminal effect of these drugs is expected to be limited. In contrast to our hypothesis, no direct inhibitory effect of these drugs on the paracellular permeability was observed, neither *ex vivo* nor *in vitro* (Chapter 5). It can however not be excluded that the drugs may affect barrier function via *e.g.* transcellular pathways or ion fluxes, as TEER values but not FITC permeation were found to be affected in the Caco-2 model. Indirect effects such as alterations in intestinal microbiota or metabolism have not been evaluated. In short, just a handful of experimental studies is currently available on the role of barrier function disturbance in MC, impeding the determination of its exact role in MC pathophysiology.

Besides smoking and drug exposure, some studies have reported on bile acid malabsorption (BAM) as a possible risk factor for MC. In MC, BAM is present in about 30-40% of MC patients.[16] The presumed role of bile acids in the pathophysiology of MC is supported by the marked clinical efficacy of bile acid binders in patients with both MC and BAM. Mechanistically, a reduced capacity to absorb bile acids in the ileum, rather than an increased colonic loss due to a higher stool frequency, is assumed to be the underlying mechanism.[17] This is supported by the therapeutic efficacy of oral budesonide. Budesonide increases the expression of the active sodium bile acid transporter (ASBT) in the murine ileum.[18] Furthermore, budesonide can normalize BAM by decreasing the hepatic bile acid synthesis (measured by C4-values). In future research, it will be of interest to further explore the relationship between BAM and MC, for instance by studying the therapeutic effect of FXR-receptor antagonists or by measuring ABST expression, faecal/serologic bile acid concentrations or markers for bile acid synthesis (*e.g.* C4) in relation to (budesonide) treatment.

Despite all abovementioned findings and assumptions, risk factors such as drug exposure, smoking and BAM are not present in all MC patients. Further studies are required to explore the exact contributory role of these risk factors as well as other underlying mechanisms and host susceptibility. Future studies should also try to assess the impact of various other (luminal) factors, *e.g.* nutrition and microbiota and metabolism. Therefore, large well-phenotyped prospective patient cohorts, preferably with collection of biomaterials are needed.

Lymphocytic versus collagenous colitis

MC comprises two subtypes, being lymphocytic colitis (LC) and collagenous colitis (CC). It is still under debate whether LC and CC are distinct clinical entities or two histological manifestations within the spectrum of MC. From a histopathological point of view, LC and CC share major characteristics such as an increased mononuclear inflammation in the lamina propria, absent crypt distortion and surface epithelium injury.[19] The features that differ between LC and CC reside within the (sub)epithelial layer: CC is characterized by a prominent sub epithelial collagen band (>10µm), while the hallmark of LC is a profound increase of intraepithelial lymphocytes (IELs) (>20 IELs / 100 epithelial cells). Based on these histological findings, LC has often been suggested to be a precursor stage of CC. However, the observation that LC patients do not differ from CC patients in age at diagnosis, argues against this assumption. Moreover, 45% of CC biopsies show increased IEL levels (>5/100 epithelial cells), while in 16% of LC cases also a slightly increased collagen band (5-10µm) can be found.[16] A transition from LC to CC, or vice versa, over time or after treatment, has been reported. Though, it is hard to prove that such transitions do actually occur and are not caused by diagnostic biases such as the

location of biopsy sampling, use of additional (immunohistochemical) stainings, tangential cutting of the biopsies, or inter-observer variability.
In Chapter 3 of this thesis, we evaluated whether the clinical presentation differs between CC and LC. For a long time, it was assumed that LC is a clinically milder form of MC with a shorter and milder disease course with less nocturnal diarrhoea, faecal incontinence and weight loss. Although we observed a longer disease course before diagnosis, more daily stools and more frequent start of medical treatment in CC as well, many other symptoms were equally disturbed between LC and CC. A recent meta-analysis[16] also showed differences in the distribution of various symptoms among both subtypes, but those were not statistically different, questioning whether LC is a preliminary variant of CC. With regard to treatment responses, it has been observed that both subtypes have a clinical response rate of >80% to oral budesonide.[20] The proportion of patients with a concomitant autoimmune disorder or celiac disease was also comparable between CC and LC, as was the prevalence of NSAID and PPI exposure (Chapter 4 and 5) and current smoking (Chapter 6 and 7).
Studies that have focussed on pathophysiological aspects of CC and LC such as genetic and immunological factors are scarce and when present, include only small populations and present opposing results. A general finding is that CC is not clearly different from LC with regard to HLA-related polymorphisms or expression of Th1, Th17, and IL-10 mRNA.[2-5] In summary, the abovementioned findings do not support strong evidence that CC and LC are two distinct entities. Unless proven otherwise, we should continue to consider CC and LC as histologically distinguishable manifestations of the same disease.

Critical appraisal of epidemiological studies in rare diseases

Several studies point to an increasing incidence of MC. In line with this observation, the number of publications on MC has been increasing from less than 10 publications annually in the early 1980s, to over 75 in 2016. The vast majority are (retrospective) observational studies, describing disease characteristics and assessing possible risk factors. A disadvantage of such studies is that they do not have the adequate design to assess causal relationships between possible risk factors (*e.g.* drug use or smoking) and the outcome (MC), as other, confounding, factors may influence the relationship. However, a statistical correction for confounders will help to increase the strength of the observed associations. For instance, in Chapter 6 and 7, smoking was taken into account as a confounding factor, when assessing the risk of *e.g.* cardiovascular comorbidities, alcohol use, or exposure to ambient air pollution and MC. Unfortunately, most confounders potentially influencing the studied associations are unknown and therefore cannot be corrected for.

Observational studies are also susceptible to various other types of methodological bias. First, the case and control population should be representative for their source population with respect to the studied exposure variables. Choosing a subset of the patients based on *e.g.* age or disease severity may introduce a selection bias. A specific form of selection-bias, *i.e.* diagnostic bias, might also be of influence. In MC, associated drugs such as PPIs, NSAIDs or SSRIs may be prescribed for or lead to certain complaints (*e.g.* diarrhoea or abdominal pain) that may increase the chance of being diagnosed with the outcome. This might increase the prevalence of drug exposure in the patient population unintentionally, distorting true associations. This may also occur when the case population has a higher *a priori* chance to receive the drug of interest, so-called prescription bias or confounding by indication. For instance, in MC patients more NSAIDs might be prescribed due to the higher prevalence of rheumatoid arthritis in this population. In addition, lack of precise data on the chronology of events can have unintended effects. Drug exposure is often based on prescription-databases such as the British CPRD or Dutch PHARMO Database Network, which generally lack information on the exact drug intake and exact start of the clinical symptoms. As a result, it is not clear whether the assessed drug exposure occurred before the first clinical manifestation, possibly introducing reverse causation or so-called protopathic bias. Inclusion of a 'lag time' (latency period) before diagnosis, in which all prescriptions of the drugs of interest are excluded, mitigates the impact of protopathic bias (Chapters 4 and 5). In addition, prescription databases lack information on exposure to over-the-counter drugs. In MC, this is concerns NSAIDs and (low-dose) PPIs. Although it is generally assumed that drugs obtained via this route are not associated with chronic, long-term intake, they may induce misclassification of exposure. However, if this misclassification is assumed to be equally distributed between cases and controls (non-differential), the impact will diminish.

With regard to the pharmaco-epidemiologic studies in this thesis, neither confounding by indication (no correction for a control group of non-MC patients with chronic diarrhoea), nor selection-bias (no biopsy revision in the CPRD-study), prescription-bias (no correction for comorbidities in PHARMO-study) or protopathic bias (no data on symptom initiation with respect to drug exposure) can be completely excluded. All current epidemiological studies on drug-induced MC are (to some extent) subject to these forms of bias. Nevertheless, the findings that have been reported are consistent between studies. Critical interpretation and assessment of the methodology and results, however, remains important.

In case-control studies, such as described in Chapter 6, recall bias is involved. This means that the degree of accuracy by which the subjects recall events or exposure, differs between the groups that were studied. Although this is inevitable in retrospective studies, it can (in part) be attenuated by equalizing the average age of both groups and the length of the recalled period, as was done in our study. Still, we cannot exclude that recall bias might be the explanation for an absence of any clear statistical differences for

some risk factors assessed in our study. Inclusion of large study populations of newly diagnosed subjects in prospective studies will further limit the impact of recall bias.

Future perspectives

MC should no longer be considered a rare condition, as reflected by the rising incidence rates.[1] The interregional and international variation in incidence rates as reported in literature, can be directly related to a varying awareness for the condition among general physicians, gastroenterologists and pathologists and possibly also to differences in risk factor exposure. Considering the impact of MC on health-related quality of life[21] it is of relevance to detect MC patients in time. In order to increase the awareness for this condition, national and international collaborative networks such as the European Microscopic Colitis Group are of value to inform physicians and to represent MC in (inter)national literature and conferences. There are high expectations of collaborative initiatives such as the PRO-MC Collaboration (Chapter 8), in which data on outcomes, exposure variables and confounders are systematically, prospectively and internationally collected. This will provide insight in general patient characteristics, the exact (long-term) disease course of the condition, and the effect of treatment strategies applied in daily clinical practice. Furthermore, such large databases provide an opportunity to study inter-observer variation in MC diagnosis and more important, to start biobanking initiatives. Especially the latter may prove to become a key for future breakthroughs in the field of microbiology, immunology and genetics. It is not expected that additional (retrospective) epidemiological studies will lead to groundbreaking insights in MC risk factors or pathophysiology.
For everyday practice, current research has shown that the association between MC and PPI / NSAID exposure is strong, as is the association with current smoking. Therefore, physicians should be aware of these factors when a diagnosis of MC is set. Cessation of drug exposure or smoking should be considered when a temporal relationship between exposure and clinical symptoms is likely. Unfortunately, any evidence on the effect of withdrawal on disease outcome is lacking and considering the high efficacy oral budesonide remains the first choice in therapy.

Conclusion

The knowledge on risk factors for MC has increased over the last decades. Smoking has been confirmed as major risk factor for MC and more details regarding patients at risk of drug-induced MC have been provided. Nevertheless, only limited progress has been made with regard to new pathophysiological insights. Although epidemiological studies help to explore new and confirm current associations between various factors and MC, it

should be acknowledged that these observational studies might be subject to bias and never prove causality. One way to overcome many forms of bias and to progress in MC research, is to establish large prospective databases such as the PRO-MC Collaboration and to complement (prospective) observational research on risk factors, long-term disease course and treatment outcomes with additional immunological, genetic and experimental studies focussing on *e.g.* barrier function *in vivo*, possible luminal toxins and microbiota.

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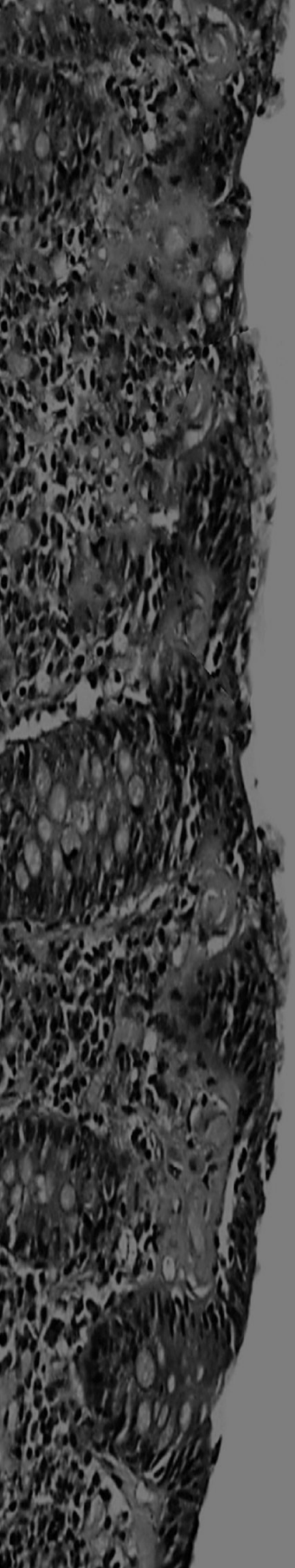


Summary

Summary

Microscopic colitis (MC) is characterized by chronic, watery, non-bloody diarrhea in combination with a normal appearance of the colon mucosa during colonoscopy and typical inflammatory changes in the biopsies. MC is applied as an umbrella term for collagenous colitis (CC) and lymphocytic colitis (LC).
Typically, the majority of MC patients is of female gender and more than 60 years old at time of diagnosis. The symptoms experienced by patients are frequent watery diarrhea, nightly defecation and abdominal pain or discomfort. Consequently, MC has a debilitating effect on patient's general well-being and daily life activities. The most effective treatment strategy is oral budesonide. Over 80% of patients achieve clinical remission within 8 weeks, although a vast majority also experiences a relapse of symptoms when treatment s stopped.
The incidence rates of MC increased markedly over the last two decades. More awareness for the condition by clinicians and pathologists is a likely contributor. Probably, changes in risk factor exposure might be involved in the increasing incidence as well. Chapter 1 gives insight in the disease characteristics of MC, including an overview of the current knowledge on MC risk factors and pathophysiology. So far, only a limited number of risk factors have been identified and the exact pathophysiological mechanisms of the disease remain unclear.
This thesis comprises several epidemiological studies on MC. First, the incidence of MC in the Netherlands was studied and the clinical characteristics of the MC population in the southeast of the Netherlands were described (Chapter 2+3). Hereafter, the risk of medication use (Chapter 4+5) and exposure to various established and unestablished (environmental) risk factors was explored (Chapter 6+7), leading to the conclusion that thorough, prospective studies are required to put MC research on a next level (Chapter 8).

To assess the incidence of MC in the Netherlands over time (Chapter 2), all Dutch MC (CC or LC) cases registered in the national registry of histo- and cytopathology (PALGA) between 2000 and 2012 were identified. The mean annual incidence rate in this period iss 3.4 per 100.000 person years, being among the lowest in Europe. In line with other studies, a significant increase in incidence rates over time was observed (from 1.9 to 5.4 per 100.000 person years between 2000 and 2012). This increase cannot be explained completely by an increase in the total number of annually performed colonoscopies. Therefore, other factors such as more awareness or varying risk factor exposure over time need to be considered.

As a clinical description of MC patients in the Netherlands is lacking, we reported on the clinical characteristics of the MC population diagnosed in the southeastern part of the Netherlands between 2000 and 2012 in Chapter 3. In general, patient characteristics

(including *e.g.* age at diagnosis, smoking status, comorbidities and clinical symptoms) were available from 553 patients and were found to be in line with international MC populations. A different clinical presentation is therefore unlikely to account for the low incidence rates in our country. As a second aim the available real-life treatment data were used to report on the outcomes of the various drugs applied as first treatment modality after diagnosis. The results show a clear prescription preference for oral budesonide over time, accompanied by a high efficacy of this drug to induce symptom remission (in 80% of patients with a completed first treatment course), compared to other treatment modalities such as mesalazine.

Chapter 4-7 of this thesis focus on various risk factors for MC aiming to find leads for underlying pathophysiological mechanisms. One of the most frequently reported risk factors so far is drug-exposure. Particularly non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs) and selective serotonin reuptake inhibitors (SSRIs) are associated with an increased risk of MC. In Chapter 4, the association between MC and these drugs was studied in more detail, based on data from the CPRD, a British general practitioner's prescription database. An almost two-fold increased risk of MC is observed in NSAID (Odds Ratio (OR) 1.86, 95% CI 1.39-2.49) and SSRI users, and a nearly four-fold increased risk in PPI exposed subjects (OR 3.37, 95% CI 2.77-4.09). In current or recent drug users, or those who continuously used the drugs in the last 4-12 months, risks are even higher. The highest risk however, is observed for co-exposure to NSAIDs and PPIs (OR 5.40, 95% CI 3.46-8.42). Additional epidemiological data were analyzed to obtain leads for possible underlying mechanisms. An idiopathic drug reaction is presumed to be less likely, as one-time and short-term use would have been expected to be associated with the highest risk. In addition, acid suppression might be a contributing factor for PPIs to induce MC, as H_2-receptor antagonists are associated with MC as well.

To confirm the strong association between NSAID/PPI co-exposure and MC, analyses were repeated in another population, with a different prescription behavior, as described in Chapter 5. Hereto, prescription data were obtained from the Dutch outpatient prescription database PHARMO. The results of these analyses confirm that concomitant use of NSAIDs and PPIs is associated with an increased risk of MC (OR 5.89, 95% CI 3.88-9.18), compared to single use (OR 2.99, 95% CI 2.31-3.88) of PPIs and NSAIDs (OR 1.96, 95% CI 1.39-2.76).
Mucosal barrier dysfunction has often been mentioned as a possible underlying mechanism, but supportive evidence for its involvement in MC pathophysiology is very limited. To study a possible direct effect of NSAIDs and PPIs or the combination thereof on the epithelial barrier function, CaCo-2 cell monolayers (an established *in vitro* culture model) were exposed to these drugs, showing no direct compromising effect on the paracellular permeability. To assess whether only genetically susceptible hosts are prone

to react upon these drugs, fresh colon biopsies of MC cases in remission and non-MC controls were exposed to NSAIDs and PPIs or both in an *ex vivo* Ussing Chamber experiment. However, no impaired paracellular permeability was observed in both groups compared to the control setting based on the flux of the permeation marker fluorescein.

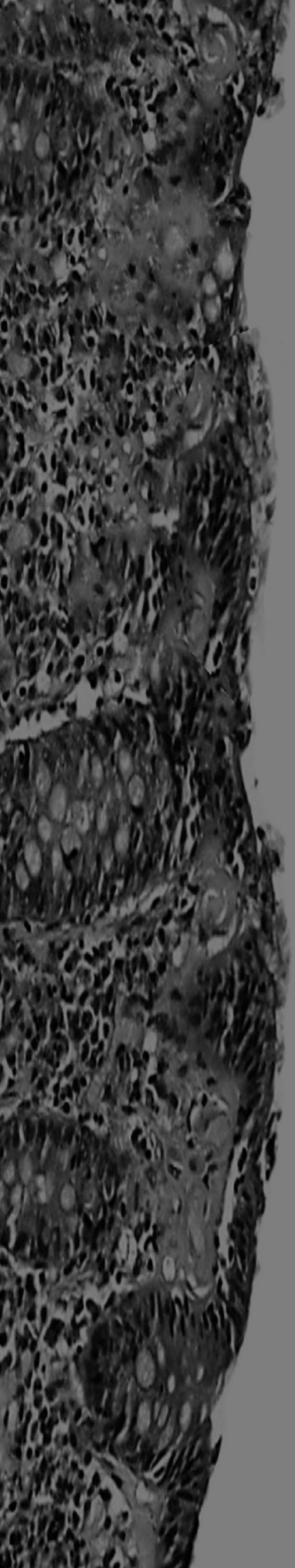
Although PPIs and NSAIDs are clearly associated with an increased risk of MC, a substantial part of the patient has not been exposed to these drugs in the recent period before diagnosis. Therefore, in Chapter 6, possible risk factors for MC other than drug exposure, were assessed. Besides the well-known risk factors such as age, gender, auto-immune diseases and smoking, also passive smoking, hormonal factors (*i.e.* because of the female predominance), general comorbidities and proxy markers for early life microbial exposure (i.e. to assess whether an increased microbial exposure in childhood might be protective to MC in later life) were studied in order to explore new leads for possible underlying pathophysiological mechanisms. Hereto, a case-control study was performed in which 171 MC cases, diagnosed between 2000-2012 in the south of the Netherlands, and 361 randomly selected controls from the same area were asked to fill out a questionnaire. Based on the results of the multivariable regression analysis current smoking (OR 6.23, 95%CI 3.10-12.49), arthrosis (OR 2.23, 95% CI 1.15-4.34) and a cardiac disorder (OR 3.31, 95% CI 1.31-8.38) are associated with MC. No association was observed for passive smoking, factors related to early life exposure to microbial antigens, rheumatoid arthritis, celiac disease or hormonal factors.

Considering both the strong association between cigarette smoking and MC and the increasing incidence over time, it was hypothesized that ambient air quality might also be a risk factor for MC. In Chapter 7, (proxy) markers for ambient air pollution, such as concentrations of various gases, proximity to major roads, population density and land use were assessed in both MC cases and randomly selected, non-MC controls in South Limburg. The individual level of exposure was determined based on the residential address, using a Geographic Information System (GIS). However, no (proxy) marker for ambient air quality, is associated with an increased risk of MC.

All aforementioned studies are based on retrospective data from regional populations. They particularly aimed to explore risk factors and leads for possible underlying pathophysiological mechanisms. To confirm the current findings and to explore other potential factors, including *e.g*. the microbiome and host genetics, it is of relevance that large prospective studies will be performed in different (foreign) populations. Considering the varying incidence rates of MC, international collaboration will be indispensable to form large well-characterised patient cohorts, preferably with biobank. One of the first initiatives for such collaborative project is the PRO-MC Collaboration. In

Chapter 8 it is described how this project was initiated. Besides the establishment of an international MC cohort, its aim is to report on the long-term disease course of MC.

A general discussion on the results presented in this thesis is provided in Chapter 9. In a detailed overview of the current knowledge on the pathophysiology of MC, a disquisition on possible underlying mechanisms for drug-induced MC is included. Furthermore, the current evidence for a disrupted colonic barrier dysfunction in MC is critically revised, concluding that this assumption is based on just a handful of experimental studies which are difficult to repeat by others. The possibilities for future research on MC risk factors and pathophysiology are explored as well, suggesting a focus on possible luminal toxins (*e.g.* bile acids), genetics and *in vivo* tests for intestinal permeability.
In the second part of this chapter we discussed that most of the clinical data on CC/LC and MC risk factors are based on retrospective, observational data, which are, inevitably, prone to various types of bias. Therefore, the results of many of the current studies might need (prospective) confirmation to increase the level of evidence and to improve the knowledge on MC pathophysiology and risk factors.



Samenvatting

Samenvatting

Microscopische colitis (MC) is een ontstekingsziekte van de dikke darm die wordt gekenmerkt door chronische, waterdunne, niet-bloedige diarree, zonder dat er sprake is van een zichtbaar afwijkend darmslijmvlies bij colonoscopie. Echter, bij pathologisch onderzoek van de darmbiopten is er wel een specifieke ontstekingsreactie zichtbaar. Op basis van het microscopisch beeld worden twee subtypes van MC onderscheiden, te weten collageneuze colitis (CC) en lymfocytaire colitis (LC) waarbij respectievelijk een verdikte collageenband onder het epitheel of verhoogd aantal lymfocyten in het epitheel aanwezig is.
Karakteristiek voor MC is dat de meerderheid van de patiënten ouder is dan 60 jaar op moment van diagnose en in driekwart van de gevallen van het vrouwelijk geslacht is. De meest voorkomende symptomen zijn frequente waterdunne diarree, nachtelijke diarree en buikpijn. Hierdoor heeft MC een duidelijke negatieve invloed op het dagelijks functioneren en algeheel welbevinden van patiënten. De meest effectieve behandeling is oraal budesonide. Binnen 8 weken bereikt meer dan 80% van de patiënten hiermee remissie van symptomen. Echter, de meerderheid van de patiënten krijgt ook weer een terugval van klachten als budesonide wordt gestopt.
De incidentie van MC is de afgelopen twee decennia duidelijk toegenomen. Meest waarschijnlijk heeft dit te maken met een toegenomen aandacht voor de ziekte onder artsen en pathologen, al kunnen veranderingen in de blootstelling aan risicofactoren gedurende deze periode ook meespelen. Hoofdstuk 1 geeft inzicht in de kenmerken van MC en biedt een overzicht van de tot nu toe bekende risicofactoren en pathofysiologie. Momenteel is slechts een beperkt aantal risicofactoren vastgesteld en ook de exacte onderliggende pathofysiologische mechanismen zijn nog niet volledig gekend.

Deze thesis omvat een aantal epidemiologische studies over MC. Allereerst is de incidentie van MC in Nederland bestudeerd en zijn de klinische kenmerken van de MC patiënten in Zuidoost-Nederland in kaart gebracht (Hoofdstuk 2+3). Vervolgens is onderzocht of medicijngebruik (Hoofdstuk 4+5) en blootstelling aan verschillende bekende en onbekende (omgevings)factoren (Hoofdstuk 6+7) het risico op MC verhoogt. Dit leidde uiteindelijk tot de conclusie dat gedegen, prospectieve studies nodig zijn om het wetenschappelijk onderzoek naar MC naar een volgend niveau te tillen (Hoofdstuk 8).

Om de incidentie van MC in Nederland vast te stellen (Hoofdstuk 2), zijn alle gevallen van MC die tussen 2000 en 2012 zijn geregistreerd in de nationale pathologieregistratie (PALGA) geïdentificeerd. De gemiddelde jaarlijkse incidentie van MC in deze periode was 3,4 nieuwe gevallen per 100.000 inwoners per jaar. Dit is nagenoeg de laagst berekende incidentie in Europa. In lijn met de resultaten van andere studies was er een aanzienlijke stijging in de incidentie tussen 2000 en 2012, namelijk van 1,9 naar 5,4 nieuwe gevallen

per 100.000 inwoners. Deze toename kan niet volledig worden verklaard door de toename in het aantal jaarlijks verrichte colonoscopieën. Andere factoren, zoals meer aandacht voor de ziekte, of een veranderde blootstelling aan risicofactoren zouden een rol kunnen spelen.

Een klinische beschrijving van de Nederlandse MC populatie is tot op heden niet beschikbaar. Hoofdstuk 3 beschrijft de patiëntkarakteristieken van de MC patiënten gediagnosticeerd tussen 2000 en 2012 in Zuidoost-Nederland. Patiëntgegevens zoals leeftijd bij diagnose, rookstatus, symptomen en aanwezige co-morbiditeit waren beschikbaar van 553 patiënten en bleken overeenkomstig met internationale patiënt-cohorten. Het is daarom onwaarschijnlijk dat een andere klachtenpresentatie van Nederlandse MC patiënten leidt tot minder diagnoses en dus een lagere incidentie. Op basis van de voorgeschreven medicatie is het effect van de medicijnen die als eerste keus werden voorgeschreven vastgesteld. Dit toont een duidelijke toename van het aantal budesonide voorschriften over de tijd, evenals een hoge effectiviteit (80%) van dit middel in vergelijking met andere medicijnen om klachten te verminderen.

Hoofdstuk 4 t/m 7 van deze thesis richten zich op verschillende risicofactoren voor MC, met als doel aanknopingspunten te vinden voor onderliggende pathofysiologische mechanismen. Een van de meest gerapporteerde risicofactoren is medicatiegebruik. Vooral niet-steroïdale pijnstillers (NSAIDs), protonpompremmers (PPIs) en selectieve serotonine heropnameremmers (SSRIs) zijn geassocieerd met een verhoogd risico op MC. In Hoofdstuk 4 zijn deze associaties in meer detail onderzocht. Hiervoor zijn data uit de Britse huisartsendatabase CPRD gebruikt. De resultaten tonen een bijna tweemaal zo hoog risico op MC bij gebruikers van NSAIDs (Odds Ratio (OR) 1.86, 95% CI 1.39-2.49) en SSRIs en zelfs een viermaal zo hoog risico bij gebruikers van PPIs (OR 3.37, 95% CI 2.77-4.09). Bij patiënten die tijdens, of tot kort voor de diagnose deze medicijnen gebruikten, is het risico zelfs nog groter, zeker als er sprake is van continu gebruik in de 4 tot 12 maanden voor diagnose. Het hoogste risico op MC is aanwezig bij patiënten die zowel NSAIDs als PPIs gelijktijdig gebruiken (OR 5.40, 95% CI 3-46-8.42). Additionele epidemiologische data zijn gebruikt om aanknopingspunten te vinden voor onderliggende mechanismen. Een idiosyncratisch medicatiereactie, waarbij sprake is van individuele overgevoeligheid voor een middel, is minder waarschijnlijk omdat eenmalig en kortdurend gebruik van deze medicijnen niet sterk geassocieerd is met een verhoogd risico. Zuurremming zou een mechanisme kunnen zijn waarop PPIs zorgen voor een verhoogde kans op MC, aangezien gebruik van andere zuurremmers (H_2-receptor antagonisten) ook geassocieerd is met MC.

Om de sterke associatie tussen gelijktijdige NSAID en PPI blootstelling en MC te bevestigen zijn de analyses herhaald in een andere populatie met een ander voorschrijfgedrag (Hoofdstuk 5). Hiertoe is bij PHARMO (een Nederlandse apothekers-

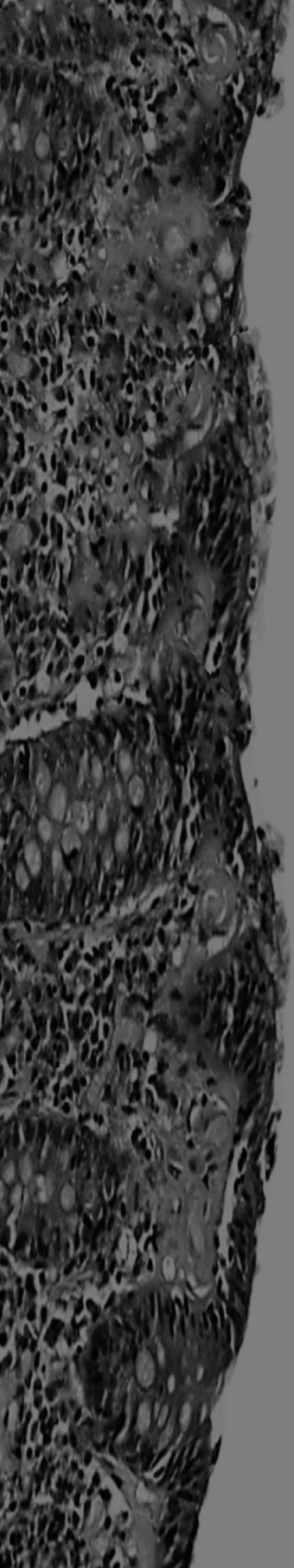
database) de medicatievoorschriften opgevraagd van zoveel mogelijk Nederlandse MC patiënten. De resultaten van de analyses bevestigen dat gelijktijdig NSAID en PPI gebruik sterk geassocieerd is met een toegenomen risico op MC (OR 5.89, 95% CI 3.88-9.18), in vergelijking met het gebruik van PPIs (OR 2.99, 95% CI 2.31-3.88) of NSAIDs (OR 1.96, 95% CI 1.39-2.76) alleen.
Dysfunctie van de slijmvliesbarrière is vaak beschreven als mogelijk onderliggend mechanisme voor MC. Ondersteunend bewijs voor deze hypothese is echter erg beperkt. Om een mogelijk direct effect van NSAIDs en/of PPIs op de slijmvliesbarrière te onderzoeken zijn CaCo-2 cellen (een gerenommeerd celkweekmodel) blootgesteld aan deze medicijnen. Een duidelijk negatief effect op de paracellulaire permeabiliteit is niet geobserveerd. Mogelijkerwijs reageren alleen MC patiënten op deze medicatie, door specifieke genetische veranderingen. Daarom zijn in een *ex vivo* Ussing kamer experiment colonbiopten van MC patiënten en gezonde controles blootgesteld aan NSAIDs, PPIs, of beiden. Echter, ook in dit experiment is geen toegenomen paracellulaire permeabiliteit waargenomen in de patiëntengroep t.o.v. de controlegroep.

Hoewel NSAIDs en PPIs sterk geassocieerd zijn met een verhoogd risico op MC, heeft een substantieel deel van de patiënten deze medicijnen nooit gebruikt in de periode voorafgaand aan de diagnose. In Hoofdstuk 6 zijn daarom nog andere mogelijke risicofactoren voor MC onderzocht. Naast de welbekende risicofactoren als leeftijd, geslacht, auto-immuunziekten en roken zijn ook passief roken, hormonale factoren (gezien de vrouwelijke predominantie), algemene co-morbiditeiten en factoren gerelateerd aan microbiële blootstelling op kinderleeftijd bestudeerd, om aanknopingspunten voor mogelijke nieuwe onderliggende pathofysiologische mechanismen te vinden. Hiervoor werd een case-controle studie opgezet waarbij 171 MC patiënten, gediagnostiseerd tussen 2000 en 2012 in Zuidoost-Nederland, en 361 willekeurig geselecteerde controlepersonen uit dezelfde regio een vragenlijst invulden. Op basis van de uitkomsten van de multivariabele regressieanalyse blijkt dat roken bij diagnose (OR 6.23, 95% CI 3.10-12.49), artrose (OR 2.23, 95% CI 1.15-4.34) en een hartaandoening (OR 3.31, 95% CI 1.31-8.38) geassocieerd zijn met een verhoogd risico op MC. Er is geen associatie met passief roken, factoren gerelateerd aan microbiële blootstelling op kinderleeftijd, reumatoïde artritis, coeliakie of hormonale factoren vastgesteld.

Gezien de sterke associatie tussen roken en MC en rees de vraag of de luchtkwaliteit in de woonomgeving wellicht ook een risicofactor zou kunnen zijn voor MC. In Hoofdstuk 7 is de blootstelling aan verschillende markers voor luchtkwaliteit (zoals gassen, nabijheid van grote wegen, bevolkingsdichtheid en landgebruik) in relatie tot MC onderzocht. Hiervoor is er een studiepopulatie gevormd bestaande uit MC patiënten en willekeurig geselecteerde inwoners zonder MC uit Zuid-Limburg. De individuele mate van blootstelling aan de verschillende markers is bepaald aan de hand van het woonadres, waarbij gebruik is gemaakt van zogenaamde Geographic Information Systems (GIS).

Uiteindelijk blijkt geen van de luchtkwaliteit gerelateerde markers geassocieerd te zijn met een verhoogd risico op MC.

Alle voorgenoemde studies zijn gebaseerd op retrospectieve data in regionale patiëntcohorten. Deze studies hebben het specifieke doel om risicofactoren en aanknopingspunten voor onderliggende pathofysiologische mechanismen te identificeren. Om de huidige bevindingen te bevestigen en om andere potentiële risicofactoren als microbiële en genetische afwijkingen te onderzoeken is het van belang dat er grote prospectieve studies worden opgezet met andere (buitenlandse) patiëntgroepen. Gezien de zeer variabele incidentiecijfers zal internationale samenwerking noodzakelijk zijn om grote, goed gekarakteriseerde patiëntcohorten te kunnen vormen of een biobank op te kunnen zetten. Een van de eerste initiatieven is de PRO-MC Collaboration. In Hoofdstuk 8 is beschreven hoe en waarom dit project is opgezet. Naast het creëren van een internationaal cohort heeft dit project ook als doel om het ziektebeloop van MC op de lange termijn te onderzoeken.
Hoofdstuk 9 omvat een algemene discussie over de resultaten van de onderzoeken in deze thesis. In een gedetailleerd overzicht is de huidige kennis over de pathofysiologie van MC beschreven, aangevuld met mogelijke onderliggende mechanismen voor medicatie geïnduceerde MC. Daarnaast is de huidige bewijslast voor een verstoorde darmbarrière in MC kritisch bekeken. Hierbij is geconcludeerd dat deze aanname is gebaseerd op slechts een handvol experimentele studies, die moeilijk te herhalen zijn. Tevens zijn de opties voor toekomstig onderzoek naar risicofactoren voor MC verkend, waarbij onderzoek naar de relatie met toxines in het colon zelf (bijv. galzouten), de relatie met genetische veranderingen en *in vivo* testen naar de darmpermeabiliteit interessante onderwerpen zijn.
In het tweede deel van Hoofdstuk 9 wordt belicht dat de meeste (klinische) data over CC/LC en risicofactoren voor MC zijn gebaseerd op retrospectieve, observationele data. Hierdoor kunnen verschillende vormen van 'bias' de resultaten hebben beïnvloed. Dit betekent dat (prospectieve) bevestiging van de huidige studies nodig is om het bewijs te versterken en de huidige kennis over de pathofysiologie en risicofactoren van MC uit te breiden.



Valorisation

Valorisation

Microscopic colitis (MC) is a chronic, inflammatory bowel disorder, characterised by watery, non-bloody diarrhoea. Typically, no endoscopic abnormalities are observed during colonoscopy, although the biopsies show clear signs of chronic inflammation. Two major subtypes of MC, *i.e.* lymphocytic colitis (LC) and collagenous colitis (CC), are distinguished based on histological findings. LC is hallmarked by an increased number of lymphocytes in the epithelium, while a thickened subepithelial collagen band is the main feature of collagenous colitis (CC). MC predominantly affects females and the average age at diagnosis is above 60 years of age. The disease can (intermittently) be present for several years. On average, patients report up to 5 bowel movements per day, mostly of watery consistency.

Worldwide, the incidence of MC has increased markedly over the last two decades. Until recently, Dutch incidence data were lacking, but the data of our national incidence study confirmed an increasing incidence in the Netherlands as well, rising from a mean annual incidence rate of 1.9 to 5.4 per 100.000 person years between 2000-2012. In line with the literature, highest incidence rates of MC in the Netherlands were observed for females (male:female ratio 1:3) and subjects above 60 years of age. The rising incidence rates can, in part, be attributed to an increased awareness for this condition among general practitioners, clinicians and pathologists, but may also be due to changes in (yet unknown) risk factors. Over time, the mean annual incidence rates are expected to rise even further, considering the increasing proportion of elderly in westernized populations together with the increased performance of colonoscopies (*e.g.* via the general practitioner and as part of population based screening on colorectal cancer) leading to more frequent detection of MC. As a consequence, clinicians will be confronted with patients suffering from MC more often. Therefore, it is of relevance that they recognize the symptoms, general patient characteristics and possible risk factors, in order to adequately identify subjects with a possible diagnosis of MC. The significant impact of MC on patients' wellbeing is an additional reason for adequate patient identification. Although MC is not associated with a clear increase in mortality or morbidity, MC clearly impairs the health-related quality of life. In MC patients with active disease, quality of life is reported to be as impaired as in patients with classical inflammatory bowel disease (IBD). This is mainly attributed to the chronic and watery aspect of the diarrhoea, which hinders patients to perform daily activities, to attend work and social events, and it makes them feel insecure about their body. This negative impact on the quality of life should be the driving force for physicians to timely identify these patients, in order to prevent for example, work absenteeism, social isolation and physical decline.
The clinical characteristics of MC such as diarrhoea, abdominal pain/discomfort, weight loss and a normal endoscopic appearance of the mucosa are however unspecific for the disease and show large overlap with symptoms of *e.g.* irritable bowel syndrome (IBS). As

the optimal treatment strategy differs largely between these entities, it is of relevance to adequately set a diagnose in patients with chronic diarrhoea. Where diarrhoea predominant IBS is mainly treated with anti-diarrheal medication, peppermint oil, antidepressants, nutritional changes, stress reduction and/or psychological interventions, MC is generally effectively treated with the oral corticosteroid budesonide. Budesonide is highly effective in the majority of patients with MC, but after cessation of therapy symptoms return in approximately 60-80% of patients. It is unclear why this happens and it stresses the importance of better understanding of potential triggers for this disease. Consequently, the majority of patients requires maintenance treatment, implying long-term exposure to corticosteroids, with an enhanced risk on *e.g.* osteoporosis and related complications. Besides oral budesonide, no evidence-based treatment strategy is available.

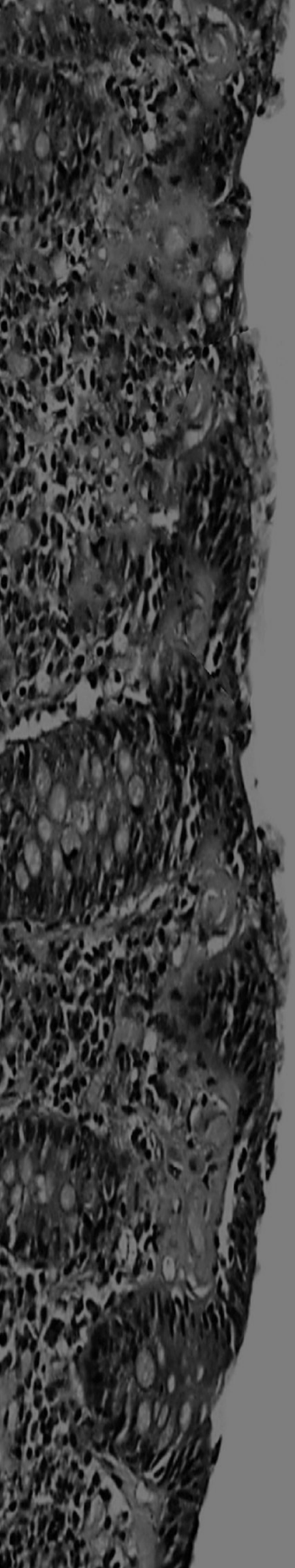
What currently impedes timely detection of patients possibly suffering of MC, is the relative ignorance of risk factors and the underlying pathophysiological mechanisms of MC. Further insight in these factors would accelerate diagnosis of MC patients and better discriminate them from those with for example IBS. Furthermore, identification of new and confirmation of presumed risk factors for MC is of relevance for implementing preventive strategies.
Currently, the number of established risk factors is limited, *i.e.* an age >60 years, female gender, smoking, the presence of autoimmune disorders such as celiac disease or rheumatoid arthritis, and the use of certain drugs. Furthermore, these factors explain only a small part of the incident cases. With this thesis we aimed to contribute to the knowledge on MC risk factors, by confirmation of the established and exploration of new potential risk factors for MC. A relevant contribution in this sense, was the confirmation that non-steroid anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) are indeed associated with an increased risk of MC. In addition, we showed that this risk rises markedly, when both drugs are used simultaneously. Interestingly, we found that the risk of MC depends on the recency and the duration of drug use, in respect to the moment of diagnosis. The risk of MC clearly decreases when the time between the last prescription and the diagnosis was more than 6 months and when the drugs had been continuously used since more than 1 year. In our opinion, this is relevant information for both clinicians and pharmacists in order to better assess the chance of a possible MC diagnosis in a patient with chronic diarrhoea, but also to determine whether drug use might play a causative role. If a causal relationship between the diarrhoeal symptoms and MC is expected based on the characteristics of the drug use, withdrawal of the drug should be considered as a first treatment strategy. This might discard, or at least postpone, the need for further anti-inflammatory treatment strategies. It should be noted that NSAIDs and PPIs are frequently prescribed by clinicians and are often freely available as over-the-counter drugs. In the Netherlands, over 10% of the national population is exposed to a PPI and over 20% to an NSAID at least once a year. In the

population above 65 years of age, the proportion of PPI users increased to up to 33%. This stresses the relevance for users and prescribers to be aware of the potential risks of these drugs. Though, it should be noted that only a small fraction of these users ultimately develops MC, which raises questions about *e.g.* genetic susceptibility and the possible pathophysiological mechanisms involved. Given the frequency of use, further studies on drug-induced MC and host-susceptibility are urgently needed. Hypothetically, a two-hit model might be applicable to MC, in which exposure to generally common drugs, leads to a clinically manifest phenotype, but only in (genetically) susceptible hosts. Genetic variations in for instance intestinal barrier function, immune function or a different composition and/or activity of the intestinal microbiota may increase the susceptibility to develop MC. However, how this relates to the onset of MC in later life is yet unclear. At the moment, a European collaborative project focussing on genetic variations in MC patients is ongoing, and patient material of our Dutch cohort will be included in this study.

Following the conclusion that NSAID and PPI exposure cannot explain all MC cases, other possible risk factors for the disease should be explored, as was done in this thesis. The only factor that was consistently associated with an increased risk of MC was smoking. Although the clinical benefit of stopping to smoke on MC symptoms is yet unknown, it has repeatedly been shown in our and other studies that smokers tend to develop their disease about 10 years earlier than non-smokers. MC is an additional condition in the long list of disorders on which smoking has a negative impact and should be considered in smoking patients presenting with chronic diarrhoea without a clear cause. Unfortunately, the underlying mechanism by which cigarette smoking causes/exacerbates MC symptoms is still unknown. Because smoking is strongly associated with MC, the role of environmental pollution in MC was also assessed, as the general compounds of cigarette smoke (nitrogen oxides, sulfoxides, particulate matter) are also present in polluted air. However, no clear associations between MC and (proxy) markers for ambient air pollution (*e.g.* the proportion of industrial area, the distance to the nearest highway, the concentration of air pollution components) were observed.
The current thesis did not focus on a potential role of the intestinal microbiota or on the immune system. It appears that these mechanisms are likely to be involved in MC. Therefore, further studies should focus on microbial changes in untreated versus treated patients, and in patients versus controls, in order to evaluate the pathophysiological role of alterations in the gut microbiome. Furthermore, additional genetic and immunological studies are urgently needed in order to further unravel the pathophysiology of the disease. Hopefully, the outcomes of such studies can be used to develop preventive strategies and more targeted treatments.

In conclusion, more awareness for MC colitis is warranted given the clear impact on patients' wellbeing and the globally and nationally increasing incidence rates. The results

of our studies provide additional and new information on clinical characteristics of Dutch MC patients and risk factors for the disease (*e.g.* NSAID and PPI exposure, smoking). Consideration or elimination of potential risk factors by clinicians will help to reduce the number of patients requiring treatment and those with a relapse after treatment. In addition, the results of this thesis might lead to better, targeted and earlier patient identification by general practitioners and clinicians. Consequently, this will not only improve the general quality of life of patients but will also reduce the costs related to absenteeism and an increased health care consumption in this generally elderly population. Although the contributed social value of the results of this thesis itself is modest, the most relevant and direct spin-off of this research project has probably been the increased regional and national attention for MC. As a consequence of this project, lectures, meetings, and collaborative projects for gastroenterologists, pathologists and GP's have been initiated in (the south of) the Netherlands and patient data have been and will be included in international collaborative studies like the PRO-MC registry. More awareness for the condition in and outside the scientific world is likely to be the major key to improved patient identification and treatment.



List of publications

List of publications

Hintaran AD, Chenault MN, **Verhaegh BPM**, Reijven PLM, Masclee AAM, Keulemans YCA. Improving nutritional status assessment in patients with chronic pancreatitis. Pancreatology 2017 (*in press*)

Verhaegh BPM, Pierik MJ, Goudkade D, Cuijpers YSMT, Masclee AAM, Jonkers DMAE. Early Life Exposure, Lifestyle, and Comorbidity as Risk Factors for Microscopic Colitis: A Case-Control Study. Inflamm Bowel Dis. 2017;23(6):1040-46

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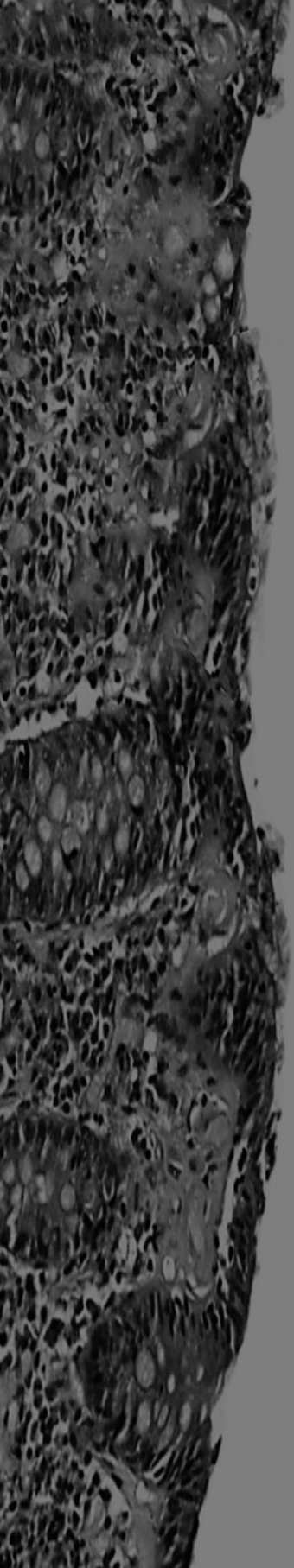

Dankwoord

Dankwoord

Hoewel het aantal promovendi die onderzoek doen naar microscopisch colitis in Europa op één hand te tellen is, heb ik ervaren dat dit zeker niet hoeft te betekenen dat je er alleen voor staat. In de afgelopen jaren heb ik het genoegen gehad om te mogen samenwerken met een fantastisch onderzoeksteam en heb ik veel lokale, nationale en internationale onderzoekers leren kennen. Een aantal van hen wil ik graag persoonlijk bedanken.

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Tijdens de ruim 4 jaar in Maastricht heeft mijn bureau altijd in de hoek van kamer 5.553 gestaan en heb ik verschillende collega's als 'roomie' gehad. In het begin heb ik het geprobeerd gezellig te maken met de toen altijd drukke en koptelefoon-minnende Fedde. Na een tijdje raakte hij gelukkig ook meer in het PhD-tempo en was er naast werk ook (ruim) tijd voor het bekijken van filmpjes, een lekkere Nespresso of het delen van fietsroutes in Zuid-Limburg. Toen Fabiënne het andere werkplekje op de kamer kreeg toebedeeld hebben we haar ook in de gezellige werksfeer kunnen betrekken. Het laatste anderhalf jaar heb ik de kamer gedeeld met Annick die me behalve met het inbedden van biopten ook kennis heeft laten maken met de meest hilarische Youtube filmpjes en vrouwen met een kort pittig kapsel. Annick, Fabiënne en Fedde dankjewel voor de fijne samenwerking, jullie hebben absoluut bijgedragen aan de positieve blik waarmee ik terugkijk op mijn promotietraject!

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In English, I would like to address **all members of the European Microscopic Colitis Group (EMCG)** and in particular **Andreas Münch, Lars Munck, Stephan Miehlke** and **Henrik Hjortswang** with whom **Marieke Pierik** and I form the PRO-MC Steering Group. As I've mentioned many times before, I've been very grateful for the opportunity to take an active role in an international collaborative network on MC. It has been very educative to collaborate with international researchers on MC and to establish an international project from scratch. But above all, I very much enjoy the personal contact between us. **Andreas**, thank you for your confidence in my contribution to the group and for your effort to keep me involved in the projects. And **Lars**, I've always appreciated your critical opinion on epidemiological and methodological matters, as well as the sincere interest you've shown in my PhD research. I'm grateful to have you as one of the opponents at my thesis defense.

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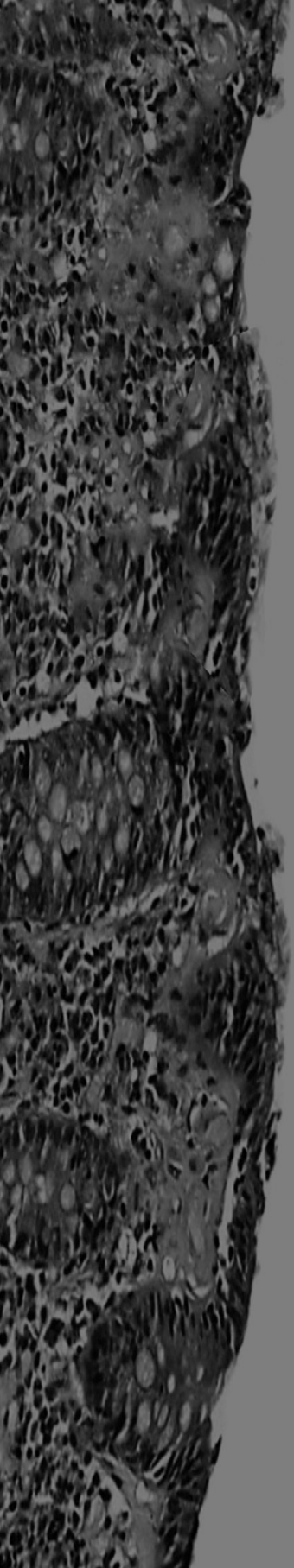

Curriculum vitae

Curriculum vitae

Bas Peter Mathijs Verhaegh, was born on October 6, 1987 in Venlo. He spent his youth in Panningen, where he obtained his gymnasium diploma cum laude at the Bouwens van der Boijecollege, in 2006. After graduation, he studied medicine at Maastricht University. During his surgical internship, his interest for gastroenterology was fuelled. Therefore, he electively followed a combined clinical and scientific internship at the gastroenterology department of the MUMC^{+}, focussing on chronic pancreatitis. In his last year of study, he continued the initiated research on chronic pancreatitis and he performed his health care participation in the department of Internal Medicine of the former Atrium Medical Centre in Heerlen. In October 2012, he started his PhD on microscopic colitis at the Division of Gastroenterology-Hepatology, within the Maastricht University research school NUTRIM, under supervision of prof. Masclee, dr. Pierik and dr. Jonkers. In February 2014, he became a member of the European Microscopic Colitis Group (EMCG). Since December 2016, he is a resident in gastroenterology and is currently working at the Department of Internal Medicine of the Viecuri Medical Center in Venlo.